## (12) UK Patent Application (19) GB

(11) 2 249 395<sub>(13)</sub>A

(43) Date of A publication 06.05.1992

(21) Application No 9120922.1

(22) Date of filing 01.10.1991

(30) Priority data (31) 07591589

(32) 02.10.1990

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(51) INT CL\* G01N 27/447, B01D 57/02

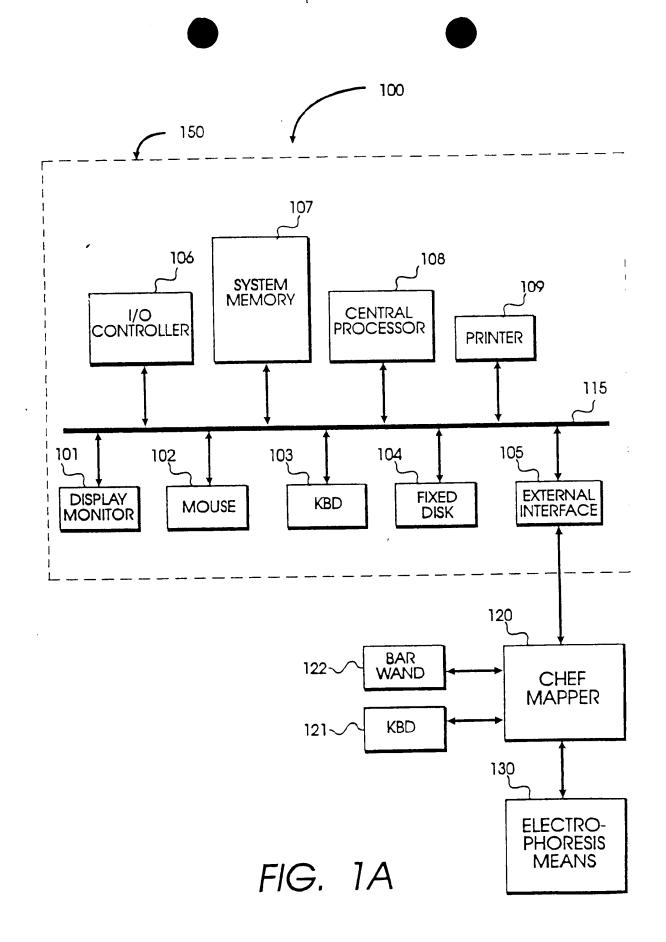
(52) UK CL (Edition K) G1N NBLE N25E1 U1S S1334

(56) Documents cited EP 0395319 A1

(58) Field of search UK CL (Edition K) G1N NBLE INT CL B01D, C07K, C25B, G01N Online databases: WPI

- (54) Automatic control system which optimises electrophoretic separation
- (57) The system optimises electrophoretic separation of macromolecules with the aid of a computer. The system maintains a knowledge base derived from calibration data obtained by separating macromolecules, in particular DNA, of known size under a variety of planned conditions chosen to optimize separations. The user enters the smallest and largest macromolecule sizes to be resolved. From this information, the system automatically generates and sets the optimal Voltage Gradient, Angle, Initial Switch Time, Final Switch Time, Forward Voltage Gradient, and Reverse Voltage Gradient parameters, thereby increasing the resolution of electrophoretic separation.

The system preferably uses the CHEF (Contour clamped Homogeneous Electric Field) system with a hexagonal electrode array.



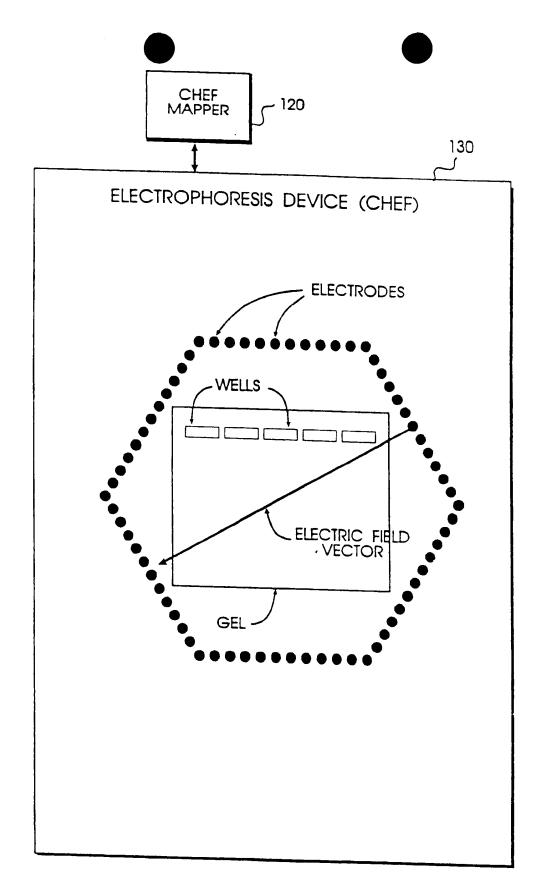
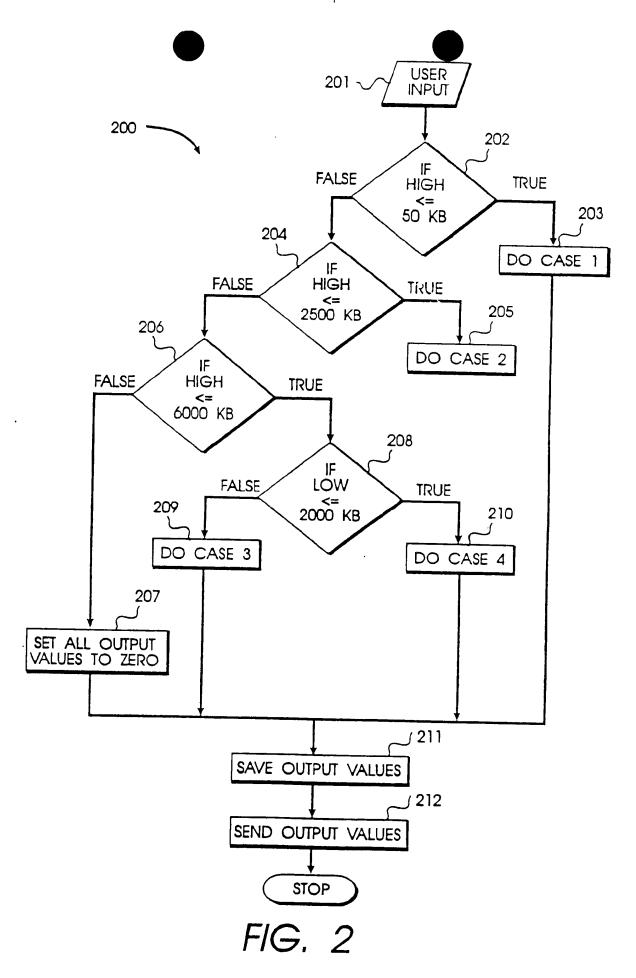


FIG. 1B

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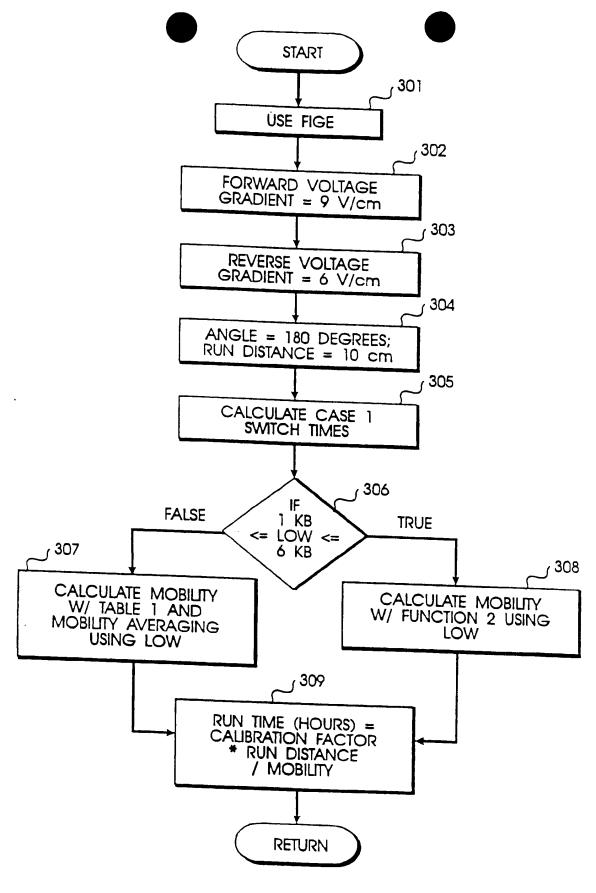


FIG. 3

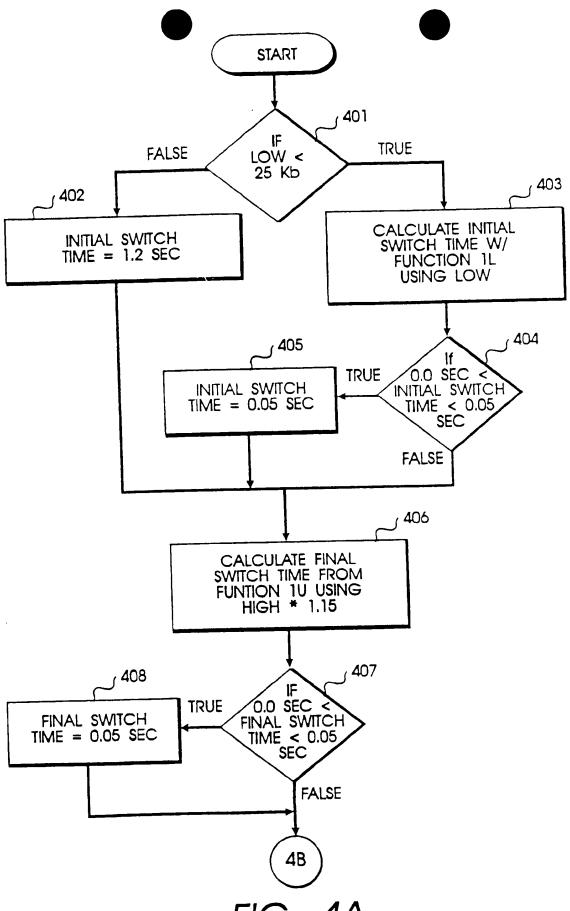
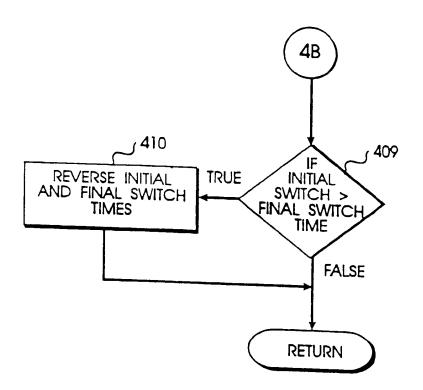


FIG. 4A



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FIG. 4B

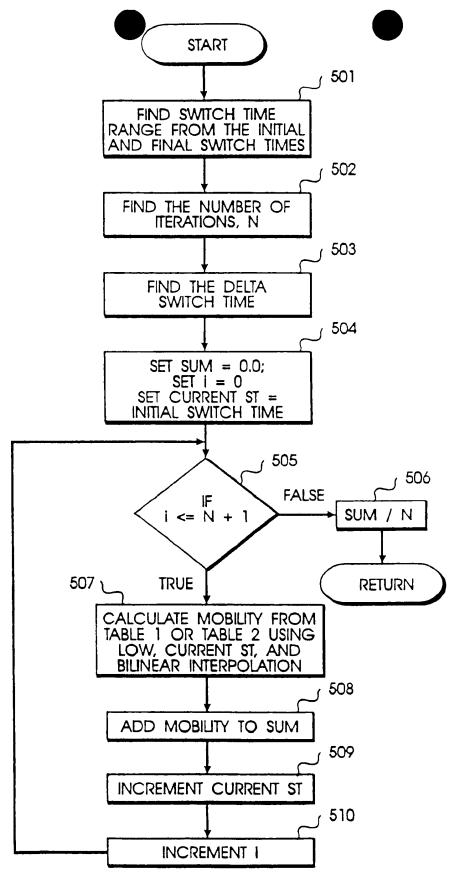


FIG. 5

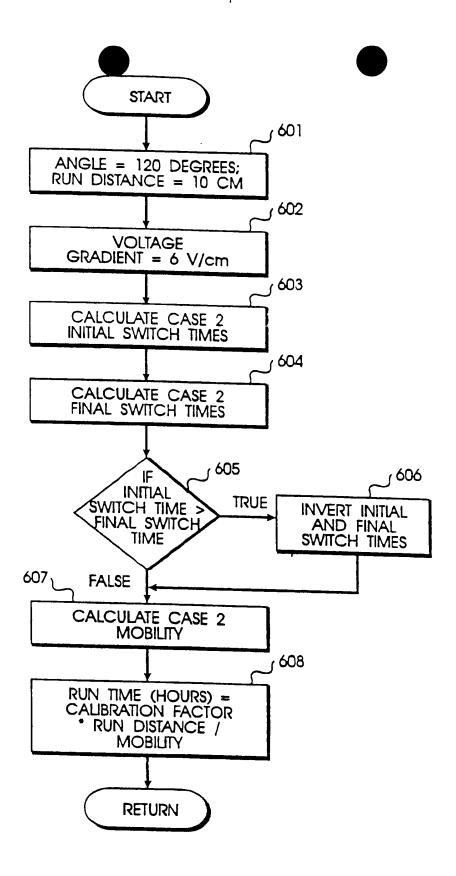


FIG. 6

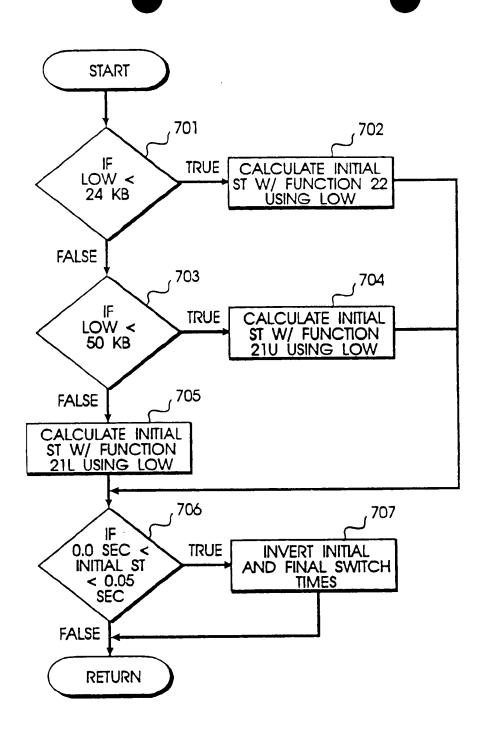


FIG. 7

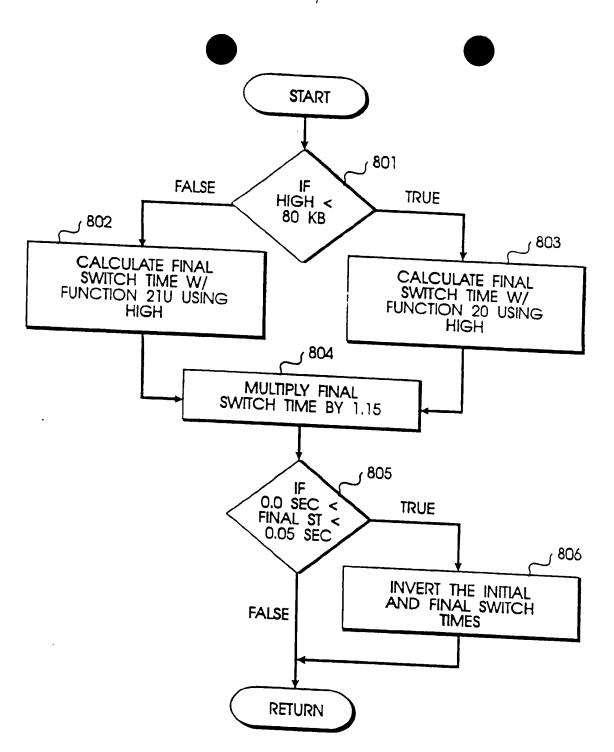


FIG. 8

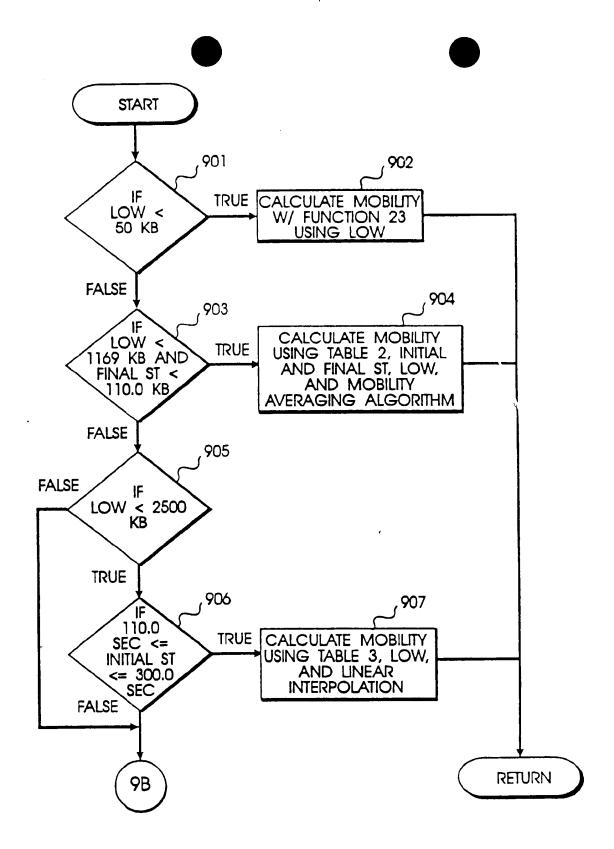


FIG. 9A

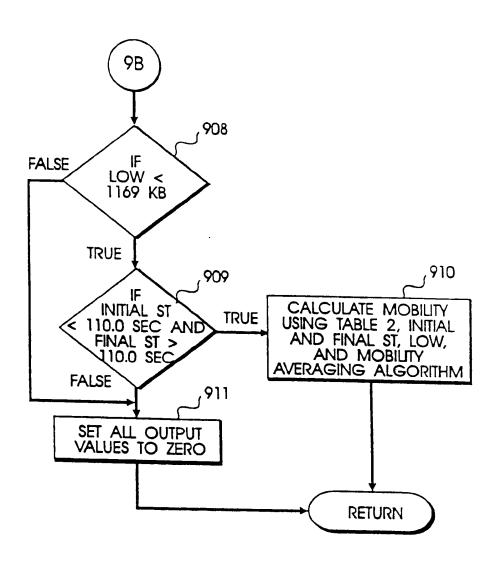


FIG. 9B

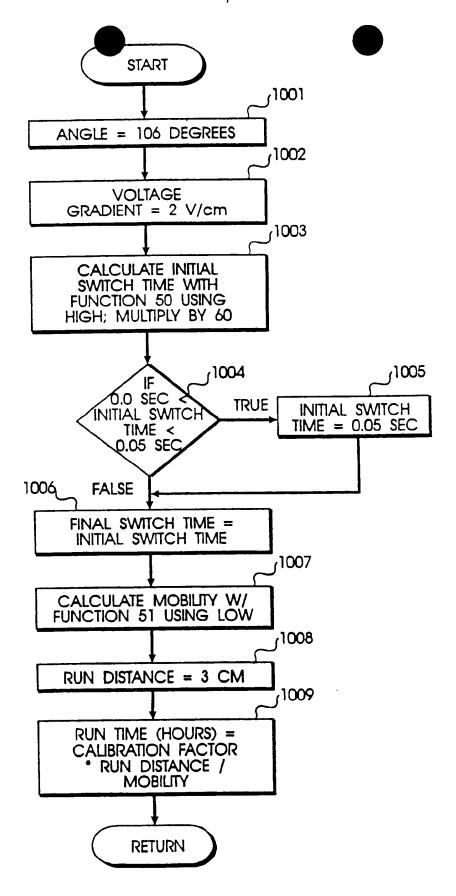


FIG. 10

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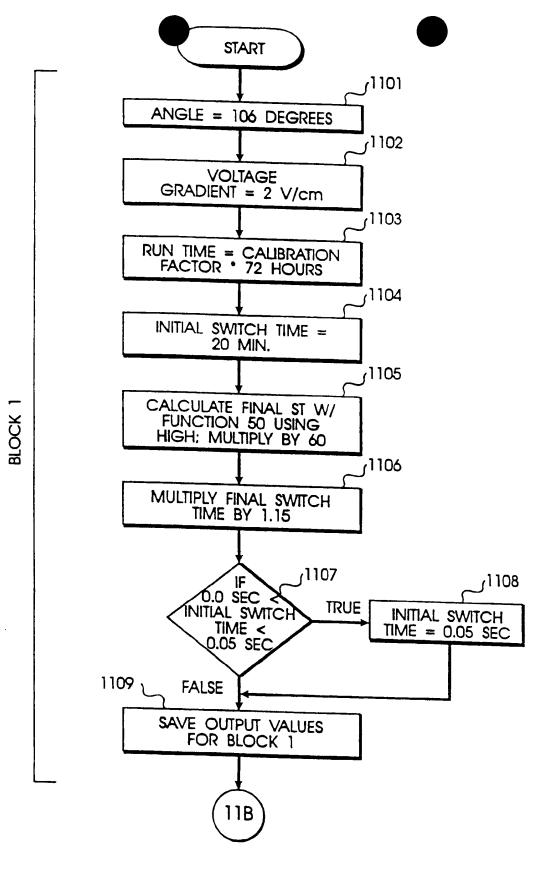


FIG. 11A

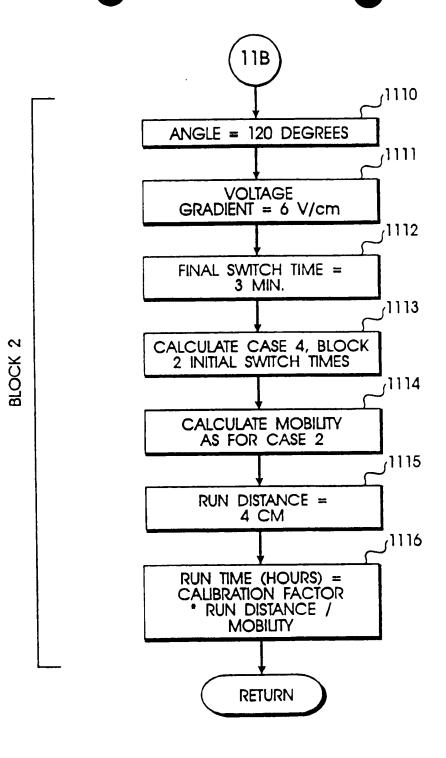


FIG. 11B

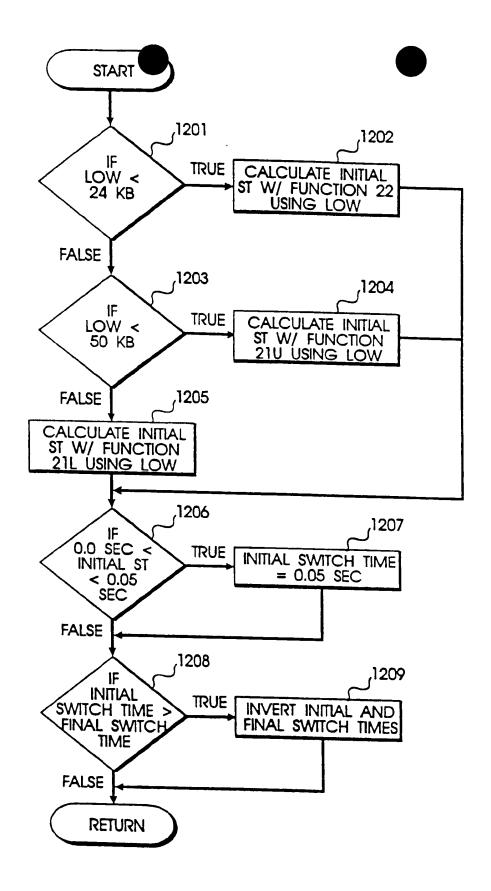


FIG. 12

# AUTOMATIC CONTROL SYSTEM FOR IMPROVED ELECTROPHORESIS BACKGROUND OF THE INVENTION

This invention relates generally to gel electrophoresis and, more particularly, to a system for controlling contour-clamped homogeneous electric field electrophoresis.

The standard method used to separate, identify, and purify DNA and other macromolecules is electrophoresis through agarose gels. This technique is capable of efficiently and rapidly resolving mixtures of DNA fragments that cannot be separated adequately by other purification technologies. A gel containing a DNA sample is subjected to an electric field. This causes the individual macromolecules in the DNA to migrate through the gel. Because different DNA fragments migrate at different rates, visible "bands" of DNA form in the gel. The location of individual DNA fragments within the gel can be read directly from the bands.

A particular DNA's characteristics, including its size, charge, and conformation, influence its rate of migration and, hence, the separation that may be achieved. For example, molecules of linear and duplex DNA travel through gel matrices at rates that are typically inversely proportional to the  $\log_{10}$  of their molecular weights. Nevertheless, DNA macromolecules of identical molecular weight but different conformations, such as closed circular, nicked circular, and linear forms, migrate through agarose gel at different rates.

In addition to DNA characteristics, electrophoretic parameters also influence the rate of migration (mobility) of macromolecules. DNA fragments of a given size migrate at different rates through gels containing different concentrations of agarose. By varying the concentration, it is possible to resolve a wide range of sizes of DNA fragments. The strength of

the applied field (voltage gradient), the ionic strength of the buffer, and the density of the superhelical twists in the closed circular form of DNA also influence migration. At low voltages, the mobility (cm/hr) of linear DNA fragments is proportional to the voltage applied. However, this linear relationship fails at higher potentials for high molecular weight fragments. Thus, the effective range of separation for a given agarose gels decreases as the voltage increases.

Improved separation of macromolecules may be achieved by optimizing certain parameters. For example, pulsed-field gel electrophoresis (PFGE), a recent advancement, allows for greater resolution of DNA fragments for a given voltage. Schwartz and Cantor teach that it is possible to resolve large DNA fragments in an agarose gel subjected alteratively to two alternately pulsing electrical fields at about 90°. (Schwartz, D., and Cantor, C., "Separation of Yeast Chromosome-sized DNAs by Pulsed-field Gradient Gel Electrophoresis," Cell 37:67, 1984.) By forcing the molecules to re-orient themselves between pulses, longer 'DNA fragments can be separated from shorter fragments. However, the induced electrical field is not uniform throughout the gel with this technique. The macromolecules tend to migrate through the gel with a mobility and trajectory that depend on where in the gel the samples are loaded. position-dependent distortion, due to the non-uniformity of the electrical field, hinders lane-to-lane comparisons of the bands.

Carle et al. describe a simple variant of PFGE, field-inversion gel electrophoresis (FIGE), in which the alternating fields are 180° apart. The polarity is reversed at intervals with a longer forward than reverse pulse time, thereby generating a net forward sample migration. This technique provides acceptable resolution up to 800 Kb (kilobases). For fragments above a certain size, however, large and small DNA molecules tend to

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migrate together. By gradually changing (ramping) the voltage over the course of the electrophoresis, one can extend the separation range before this occurs. However, a set of parameters appropriate for the size of the molecule to separate must be selected at the outset.

(See, Carle, G., Frank, M., and Olson, M.,
"Electrophoretic Separations of Large DNA Molecules by Periodic Conversion of the Electric Field," Science 232:65, 1986.)

Gardiner and Patterson describe a transverse alternating field electrophoresis (TAFE) system in which the gel is oriented vertically and the usual four-electrode array is not placed in the plane of the gel but in front and in back of it. (Gardiner, K., Laas, W., and Patterson, D., "Fractionation of Large Mammalian DNA Restriction Fragments Using Vertical Pulsed-Field Gradient Gel Electrophoresis," Som. Cell Molecular Genetics 12:185, 1986.)

Chu et al. teach that the critical factor in resolving large DNA fragments is keeping the angle between the two directions of the alternating electrical field greater than 90°. (Chu, G., Vollrath, D., and Davis, R., "Separation of Large DNA Molecules by Contourclamped Homogeneous Electric Fields," Science 234:1582, A contour-clamped homogeneous electric fields or CHEF system employs a hexagonal array with pairs of electrodes placed at 120°. Using transistors to force the electrodes to their ideal voltages, the field is clamped. Increased resolution is obtained without comigration of samples. CHEF-DR II (BIO-RAD, Richmond, California) employs this technique and is used extensively for the separation of large DNA molecules. (U.S. Patent Number 4,878,008, Contour-clamped homogeneous electric field generator, issued to C. Ragsdale.)

Current systems, however, require the user to manually determine and enter the parameters for the

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electrophoretic operation. This technique is inefficient, requiring much trial and error by the user. Specifically, this approach fails to take advantage of a knowledge base that may be constructed from the 5 electrophoretic separation of DNA standards under a variety of set conditions. The invention provides novel techniques and apparatus which fulfill these and other needs. 10 The present invention provides a system for improving the electrophoretic separation of macromolecules with the aid of a computer. The system maintains a knowledge base derived from calibration data obtained by separating macromolecules, in particular DNA, of known size under a variety of planned conditions 15 chosen to optimize separations. In a preferred embodiment, the knowledge base is embodied in constants, functions, and tables as part of a CHEF Mapper Program. In operation, the user enters the smallest and 20 largest DNA sizes to be resolved. From this information, the system automatically calibrates an electrophoresis device with the optimal Voltage Gradient, Angle, Initial Switch Time, Final Switch Time, Forward Voltage Gradient, and Reverse Voltage Gradient parameters, thereby 25 increasing the resolution of electrophoresis. Fig. 1A is a block diagram of the system of the present invention. Fig. 1B is a block diagram of the Mapper and 30 the electrophoresis device. Fig. 2 is a flowchart of the CHEF Mapper Program. Fig. 3 is a flowchart of Case 1. Figs. 4A-B are a flowchart of the determination 35 of the Switch Times for Case 1. Fig. 5 is a flowchart of the Mobility Averaging Routine.

Fig. 6 is a flowchart of Case 2.

Fig. 7 is a flowchart of the determination of the Initial Switch Times for Case 2.

Fig. 8 is a flowchart of the determination of the Final Switch Times for Case 2.

Fig. 9 is a flowchart of the Mobility determination for Case 2.

Fig. 10 is a flowchart of Case 3.

Figs. 11A-B are a flowchart of Case 4.

Fig. 12 is a flowchart of the determination of the Initial Switch Times for Case 4.

#### Overview

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apparatus which work in conjunction with an electrophoresis means to improve the separation and purification of macromolecules, particularly DNA.

According to the invention, a user enters information about the macromolecular sample to be separated.

Parameters which optimize separation are then determined. Finally, the electrophoresis means is calibrated using these parameters.

Referring to Fig. 1A, the present invention may be embodied in a system such as the system 100, which comprises a computer system 150, a CHEF Mapper 120, and an electrophoresis device or means 130. Computer system 150 further comprises a central processor 108, a system memory 107, an input means or keyboard 103, a mouse 102, a fixed disk 104, a display monitor 101, an external interface 105, a printer 109, and an input/output controller 106. The components of computer system 150 are coupled to a system bus 115, thereby providing a link between all components. In a preferred embodiment, an appropriately programmed IBM compatible personal computer is used (International Business Machines, Armonk, NY) running under Microsoft Windows (Microsoft, Redmond, WA).

Referring to Fig. 1B, CHEF Mapper 120 and electrophoretic device 130 are further illustrated. Mapper 120 is coupled to device 130 and, thus, can directly calibrate device 130 with optimized parameters. In a preferred embodiment, device 130 is an electrophoresis means employing CHEF techniques. Macromolecule samples, which are initially placed in wells, migrate across a gel in response to an electric field. More specifically, the samples migrate under the influence of an electric field vector generated by a plurality of electrodes.

In one embodiment, CHEF Mapper 120 is also coupled to external interface 105, such as a RS-232 serial port, thereby allowing Mapper 120 to receive output from system 150 which, in turn, is used to calibrate electrophoresis device 130. In another embodiment, CHEF Mapper 120 is coupled to its own input device, such as a keyboard 121 and/or a bar wand (bar code reader) 122, thereby allowing Mapper 120 to receive information directly from the user, from alternative input formats, or from other systems. As shown in Fig. 1A, Mapper 120 can also be coupled to external interface 105 and to keyboard 121 and bar wand 122, all at the same time.

System 100 operates in a fully automatic mode: the user enters the lowest and highest fragment sizes (in kilobases) of macromolecules (typically DNA) expected to be separated. A knowledge base of relationships discovered by applicants is provided which enables system 100 to automatically determine the optimal parameters: Forward Voltage Gradient, Reverse Voltage Gradient, Voltage Gradient, Angle, Initial Switch Time, Final Switch Time, and Run Duration, as well as the best electrophoretic technique. Thus, maximum macromolecule separation can be achieved.

In an alternative embodiment, system 100 operates in a semi-automatic mode: the user can specify

additional parameters, including agarose type, agarose

additional parameters, including agarose type, agarose concentration, buffer type, buffer concentration, and temperature. These values are then used, in conjunction with the DNA sizes, to determine the optimal parameters.

The individual parameters for system 100 will now be described in further detail. The Forward Voltage Gradient expresses the voltage differential per distance (V/cm) which is used during the forward pulse of FIGE. The Reverse Voltage Gradient, on the other hand, is the voltage differential per distance (V/cm) for the reverse pulse. The Voltage Gradient is simply the difference between the Forward and Reverse Voltage Gradients.

Switch Time is the duration that electrophoretic device 130 remains at a particular state (before switching again). Thus, this is the period of time that the system maintains a particular set of vectors (voltage gradient at a particular angle). The Switch Time is ramped (varied) during the course of electrophoresis. Preferrably, the Switch Time is ramped in an increasing fashion. More preferrably, the Switch Time is ramped in a non-linear, increasing fashion. preferred embodiment, the Switch Time is ramped logarithmically. In an alternative embodiment, the Switch Time is ramped hyperbolically. In yet another embodiment, the user specifies the ramping by defining a curve on a coordinate system, for example, a cartesian coordinate system where X is the normalized Run Duration and Y is the normalized difference between Initial and Final Switch Times. Regardless of the ramping technique employed, it is preferable to divide the Switch Time into the Initial and the Final Switch Times indicating the upper and lower limits of the Switch Time ramp.

Angle and Run Duration retain their usual connotations. The Angle is the angle maintained between the alternating fields and the sample. Historically, forward or down is defined as angle equal to 0°; for reverse or up, angle equals 180°. Finally, Run Duration

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is the period of time that the electrophoresis is carried out by electrophoresis device 130.

Since a user may be familiar with a particular set of terminology, three different program types are

set of terminology, three different program types are used in a preferred embodiment: 1) FIGE, 2) Two-State CHEF, and 3) Multi-State CHEF. This grouping aids the user, for example, in viewing run parameters. The FIGE has only forward (angle = 0°) and reverse (angle = 180°) pulses. In the Multi-State CHEF or program type, forward occurs when angle equals 0°, but there are also offsets, either positive or negative. For example, 60° to one side has a corresponding offset of -60° so that the net load is in a forward direction. Two-State CHEF is similar to Multi-State CHEF except it uses an included angle. Thus, for 60° and -60° angles, there would be an included angle of 120°.

The three program types have subset/superset relationships. All of the program types can be described by the Multi-State CHEF program type. This program type may include up to eight blocks, each block capable of including up to fifteen states (véctors). The Two-State program type includes one block with two symetrical states. All values for each parameter in both vectors are identical except for angle. The angles are symetrical so that they sum to zero (forward direction).

FIGE is also a special case of the Multi-State CHEF. It also consists of one block with two vectors. However, these vectors are not symetrical. One vector always has an angle of 0° (forward), while the other has an angle of 180° (reverse).

For standard conditions in the fully automatic mode, the following values are assumed for small DNA sizes (< 2500 Kb):

1) agarose type = "Low EEO" (industry
standard),

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- 2) agarose concentration = 1.0%,
- 3) buffer type = TBE (tris-borate EDTA),
- 4) buffer concentration = 0.5% TBE, and
- 5) buffer temperature = 14 degrees Celsius.

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The following standard conditions are assumed for larger DNA sizes):

1) agarose type = Low EEO,

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- 2) agarose concentration = 0.8%,
- 3) buffer type = TAE (tris-acetate EDTA),
- 4) buffer concentration = 1.0X TAE, and
- 5) buffer temperature = 14 degrees Celsius.

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The knowledge base of system 100 is derived from calibration data obtained by separating DNA molecules of known size under a variety of planned conditions chosen to optimize separations. In a preferred embodiment, the knowledge base is embodied in constants, functions, and tables as part of a CHEF Mapper Program. The functions were obtained with polynomial and curvilinear regressions. The surfaces generated were fitted with a distance weighted moving least squares procedure performed on normalized data. (See, Lancaster, Peter, and Salkauskas, Curve and Surface Fitting: An Introduction, Academic Press, 1974; McLain, Drawing Contours from Arbitrary Data Points, Comput. J., 17:318-324, 1974.) Both the functions and the fitted surfaces are used for interpolation and in some cases for

#### Effect of DNA Size

extrapolation.

The fully automatic operation of system 100 is divided into four different cases, the actual case being determined by the maximum and minimum DNA sizes entered by the user. In a preferred embodiment, three ranges of DNA sizes are defined: (1) 1-50 Kb, (2) 50-2200 Kb, and (3) greater than 2500 Kb. Each range has its own

particular set of macromolecular characterist

particular set of macromolecular characteristics which can be exploited to optimize separation.

For DNA fragments of 1-50 Kb, for example, movement in a pulsed field is a logarithmic function of its size. This is in contrast to larger DNA fragments which have a mobility which is linear to their size. Fragments of different sizes do not respond identically to fields of different strengths. Larger fragments, e.g., 30-50 Kb, move relatively faster at 9 V/cm than do fragments of about 1-25 Kb. Different field strengths can be used to reduce the large spacing (distance between DNA bands) that occurs with the smaller bands (less than 10 Kb). As a result, more uniform spacing occurs over the entire range. Thus, a preferred embodiment employs a field-inversion technique which uses different voltages for the forward and reverse fields for fragments below 50 Kb to spread out fragments in a more uniform manner (compared to pulsed fields of identical voltages).

DNA between 50 and 2500 Kb represent the range that was first described by Schwartz and Cantor. Most pulsed-field gel separations are designed to separate DNA within this range.

To separate large fragments of DNA, the field strength must be reduced to prevent the large molecules from getting tangled or broken in the gel. However, these low fields strengths necessitate extremely long run times. Since the angle between the two fields influences the mobility of the DNA, run times may be decreased by reducing the angle between fields.

### System Operation

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The operation of the system 100 will now be described in detail. Fig. 2 is an overview of the CHEF Mapper Program 200, which is a set of procedures or steps stored in system memory 107 for instructing central processor 108. In another embodiment, CHEF Mapper Program 200 is stored in a firmware, e.g., a ROM, and

coupled either to system bus 115 or directly to Mapper 120.

enters input parameters into keyboard 103 and/or mouse 102; if program 200 is directly coupled to Mapper 120, parameters are more preferrably enter into keyboard 121 or bar wand 122. For automatic mode, the user need only enter the minimum DNA size and the maximum DNA size of the sample to be separated. For the non-automatic mode, the user also enters the agarose type, agarose concentration, buffer type, buffer concentration, and temperature. Regardless of the mode, the input parameters are stored in a table, InputParms, located in system memory 107 and/or on fixed disk 104.

In step 202, if the maximum DNA size entered ("High") is less than or equal to 50 Kb, then the procedure goes to step 203 to perform Case 1. Otherwise, the procedure goes to step 204. If High is less than or equal to 2500 Kb in step 204, then the procedure goes to step 205 to perform Case 2. Otherwise, the procedure goes to step 206. If High is less than or equal to 6000 Kb in step 206, then the procedure goes to step 208 to determine if the minimum DNA size entered (Low) is less than or equal to 2000 Kb. If this is true, then the routine goes to perform Case 4 in step 210. Otherwise, the procedure goes to perform Case 3 in step 209.

However, if High is not less than or equal to 6000 Kb in step 206, then in step 207 all output values are set equal to zero. After this step, or after Cases 1-4 are performed, the procedure goes to step 211 and saves all the output values in an OutputParms table. In step 212, these values are sent to the CHEF Mapper 120, either directly by program 200 or via external interface 105. CHEF Mapper 120, in turn, calibrates electrophoresis device 130 with the optimum Angle, Initial Switch Time,

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Final Switch Time, Voltage, Run Duration, Forward Voltage, and Reverse Voltage.

Individual Cases

The individual cases within CHEF Mapper Program 200 which form the knowledge base will now be described. Fig. 3 illustrates the steps for Case 1, that is, when High is less than or equal to 50 Kb. In step 301, field-inversion gel electrophoresis (FIGE) technique is chosen. In step 302, the Forward Voltage Gradient is set equal to 9 V/cm. In step 303, the Reverse Voltage Gradient is set equal to 6 V/cm. In step 304, the Angle is set equal to 180°, and the Run Distance is set equal to 10 cm. In step 305, the Switch Times are determined.

Figs. 4A-B are a flowchart which illustrates the steps of determining the Switch Times. In step 401, if Low is less than 25 Kb, then in step 402 the Initial Switch Time is set equal to 1.2 seconds. Otherwise, the procedure goes to step 403 to calculate the Initial Switch Time using the following relationship: (Function 1L)

 $Y = 0.0 + 0.015797 X + 0.001365 X^2$ 

where X is the value of Low, and Y is the Switch Time in seconds. In step 404, if the Initial Switch Time is between 0.0 seconds and 0.05 seconds, then the Initial Switch Time is set equal to 0.05 seconds in step 405. Otherwise, step 405 is skipped. In step 406, the Final Switch Time is determined from the following relationship:

30 (Function 1U)

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 $Y = 0.0 + 0.009166 X + 0.000137 X^2$ 

where X is the value of High and Y is the Switch Time in seconds. The result of Y is multiplied by 1.15, an empirically derived adjustment factor. In step 407, if the Final Switch Time is between 0.0 seconds and 0.05 seconds, then in step 408 the Final Switch Time is

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set equal to 0.05 seconds. Otherwise, step 408 is skipped. In step 409, if the Initial Switch Time is greater than the Final Switch Time, then in step 410 the values for Initial and Final Switch Times are reversed (swapped for one another). Otherwise, step 410 is skipped. The subroutine returns, thus concluding step 305.

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In step 306 if Low is between 1 Kb and 6 Kb or equal to either, then in step 308 the Mobility (cm/hr) is determined using the following relationship: (Function 2)

 $Y = 0.65426181 - 0.0838514428 X + 0.0060879216 X^2$ ,

where X is the value for Low. However, if Low is not between the range of step 306, then in step 307 the Mobility (cm/hr) is determined from Table 1 (Appendix) and the Mobility Averaging Routine.

Fig. 5 illustrates the steps of the Mobility Averaging Routine. In step 501, the Switch Time Range is determined from the Initial and Final Switch Times. step 502, the number of iterations, N, is determined by dividing the Switch Time Range by five and rounding to the nearest integer. In step 503, the Delta Switch Time is determined by dividing the Switch Time Range by N. In step 504, the following constants are set: sum = 0.0, i = 0, Current Switch Time = Initial Switch Time. step 505, i is tested to count N. If i is not less than or equal to N + 1, then the routine divides sum by N in step 506 and stops, thus returning to step 307. Otherwise, the routine continues to step 507 to calculate a Mobility from either Table 1 or Table 2 (Appendix) using Low as the values of DNA size, current Switch Time as the Switch Time, and bilinear interpolation. step 508, the Mobility is added to the sum. In step 509, the Current Switch Time is incremented. In step 510, i

is incremented, and the procedure loops back to step 505.

After completion of the Mobility Averaging Routine in step 307, the Run Time is determined in step 309 by dividing the Run Distance by Mobility and multiplying by a Calibration Factor. Slight variations in any one of several factors, e.g., water quality and laboratory technique, can significantly effect the operation of system 100. To minimize these variations, the user adjusts the Calibration Factor based on his or her experience with the system. The output of program 200 is adjusted accordingly. The Case 1 routine concludes by returning to step 203.

Fig. 6 illustrates the steps of Case 2. In step 601, Angle is set equal to 120°, and Run Distance is set equal to 10 cm. In step 602, the Voltage Gradient is set equal to 6 V/cm. In step 603, Initial Switch Times for Case 2 are determined. Fig. 7 illustrates the steps involved. In step 701, if Low is less than 24 Kb, then in step 702 the Initial Switch Time is determined using the following relationship: (Function 22)

 $Y = 0.0 + 0.072277 X - 0.008314 X^2 + 0.000581 X^3$ 

where X is Low, and Y is the Switch Time. The procedure then jumps to step 706. However, if Low is not less than 24 Kb in step 701, then in step 703 Low is examined to see if it is less than 50 Kb. If this is true, then in step 704 the Initial Switch Time is determined using the

following relationship:
(Function 21U)

Y = -0.0701979 + 0.074324119 X + 0.000007336094 X<sup>2</sup>

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where X is Low and Y is the Switch Time. The routine continues on to step 706, However, if Low is not less than 50 Kb in step 703, then in step 705 the Initial Switch Time is determined using the following relationship:

(Function 21L)

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 $Y = 0.95604 + 0.11662974 X - 0.00000574226 X^2$ ,

where X is Low and Y is the Switch Time. In step 706, if the Initial Switch Time is between 0.0 seconds and 0.05 seconds, then in step 707 the values for the Initial and Final Switch Times are inverted or reversed. The routine concludes, thus concluding step 603 of the calling routine.

In step 604, the Final Switch Time is determined. Fig. 8 illustrates the steps of this method. In step 801, if High is less than 80 Kb, then in step 802 the Final Switch Time is determined using the following relationship:

20 (Function 21U)

x<sup>2</sup>

Y = -0.0701979 + 0.074324119 X + 0.000007336094

where X is High and Y is the Switch Time. However, if High is less than 80 Kb in step 801, then the procedure goes to step 803 to calculate the Final Switch Time using the following relationship:

(Function 20)  $Y = 0.0 + 0.007605 X + 0.000686 X^2$ 

where X is High and Y is the Switch Time (sec). The procedure continues on to step 804, where the Final Switch Time is multiplied by 1.15. In step 805, if the Final Switch Time is between 0.0 seconds and 0.05 seconds, then in step 806 the values for the Initial and Final Switch Times are inverted. The procedure

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concludes, thus concluding step 604 of the calling routine.

In step 605, if the Initial Switch Time is greater than the Final Switch Time, then in step 606 the values for the Initial and Final Switch Times are inverted. Otherwise, step 606 is skipped. In step 607, the Mobility for Case 2 is determined.

Fig. 9 illustrates the process involved in step 607. In step 901, if Low is less than 50 Kb, then in step 902 the Mobility (cm/hr) is determined using the following relationship: (Function 23)

#### $Y = 1.0124518 \times 0.4094122386$

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where X is Low and Y is the Mobility. In step 903, if Low is less than 169 Kb and the Final Switch Time is less than 110.0, then in step 904 Mobility (cm/hr) is determined using Table 2 (Appendix), Initial and Final Switch Times, Low as the DNA size, and the Mobility Averaging Routine (Fig. 5). Then, the procedure concludes by returning.

However, if step 903 is false, then the procedure goes to step 905 to test whether Low is less than 2500 Kb. If this is true, then in step 906 the Initial Switch Time is tested to see if it is between 110.0 and 300.0 seconds. If this is true, then at step 907 the Mobility (cm/hr) is determined using Table 3 (Appendix), the value of Low as the DNA size, and linear interpolation. Thereafter, the procedure concludes, thus concluding step 607 of the calling routine.

However, if the known DNA size is not less than 2500 Kb in step 905, then steps 906 and 907 are skipped. Furthermore, if step 906 is false, then step 907 is skipped. In step 908, Low is tested to see whether it is less than 1169 Kb. If this is true, then in step 909 if the Initial Switch Time is less than 110.0 seconds and

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the Final Switch Time is greater than 110.0 seconds, then the procedure goes to step 910 to calculate Mobility using Table 2 (Appendix), Initial and Final Switch Times, Low as the DNA size, and the Mobility Averaging procedure. Then, the routine concludes by returning.

However, if Low is not less than 1169 Kb in step 908, then steps 909 and 910 are skipped. Also, if step 909 is false, then step 910 is skipped. Under these circumstances, the routine arrives at step 911 where it sets all output values to zero before concluding step 607.

In step 608, the Run Time is set equal to the Calibration Factor multiplied by the Run Distance and divided by the Mobility. The Case 2 procedure concludes and, thus, concludes step 205.

Fig. 10 illustrates the steps for the Case 3 procedure, which is called at step 209 (Fig. 2). In step 1001, Angle is set equal to 106°. In step 1002, the Voltage Gradient is set equal to 2 V/cm. In step 1003, the Initial Switch Time is determined using the following equation:

(Function 50)

Y = 8.6 + 0.0053 X

where X is High multiplied by 60 and Y is the Switch Time.

In step 1004, if the Initial Switch Time is between 0.0 and 0.05 seconds, then in step 1005, the Initial Switch Time is set equal to 0.05 seconds. Otherwise, step 1005 is skipped. In step 1006, the Final Switch Time is set equal to the Initial Switch Time. In step 1007, the Mobility (cm/hr) is determined by using the following relationship:

(Function 51)

 $Y = 0.047 + 0.00000251 X + -1.56 10^9 X^2$ 

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where X is Low and Y is the Mobility. In step 1008, the Run Distance is set equal to 3 cm. In step 1009, the Run

Time is set equal to the Calibration Factor multiplied by the Run Distance and divided by Mobility. The Case 3 concludes and, thus, concludes step 209.

Figs. 11A-B illustrate the steps of Case 4, called at step 210 (Fig. 2). In step 1101, Angle is set equal to 106°. In step 1102, the Voltage Gradient is set equal to 2 V/cm. In step 1103, the Run Time is set equal to the Calibration Factor multiplied by 72 hours. In step 1104, the Initial Switch Time is set equal to 20 minutes. In step 1105, the Final Switch Time is determined using the following relationship: (Function 50)

Y = 8.6 + 0.0053 X

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where X is High multiplied by 60 and Y is Mobility. In step 1106, the Final Switch Time is multiplied by 1.15. In step 1107, if the Final Switch Time is between 0.0 and 0.05 seconds, then in step 1108, the Final Switch Time is set equal to 0.05 seconds. Otherwise, step 1108 is skipped. In step 1109, the output values for block 1 are saved.

In steps 1110-1111, the Angle is set equal to 120° and the Voltage Gradient is set equal to 6 V/cm. In step 1112, the Final Switch Time is set equal to 3 minutes. In step 1113, Initial Switch Times are determined.

Fig. 12 illustrates the method for determining the Initial Switch Times for Case 4. In step 1201, if low is less than 24 Kb, then in step 1202 the Initial Switch Time is determined using the following relationship: (Function 22)

 $Y = 0.0 + 0.072277 X - 0.008314 X^2 + 0.000581 X^3$ 

where X is the value of Low and Y is the Switch Time. The routine then continues on to step 1206. However, if Low is not less than 24 Kb in step 1201, then the procedure

19 Loss to the loss to loss to

goes to step 1203 to test whether Low is less than 50 Kb. If this is true, then in step 1204 the Initial Switch Time is determined using the following relationship: (Function 21U)

 $Y = -0.0701979 + 0.074324119 X + 0.000007336094 X^2$ ,

where X is the value of Low and Y is the Switch Time. The procedure continues on to step 1206. However, if Low is not less than 50 Kb in step 1203, then the Initial Switch Time is determined in step 1205 by using the following relationship:

(Function 21L)

 $Y = 0.95604 + 0.11662974 X - 0.00000574226 X^2$ 

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where X is the value of Low and Y is the Switch Time.

The procedure continues on to step 1206 to determine if the Initial Switch Time is between 0.0 and 0.05 seconds. If this is true, then in step 1207 the Initial Switch Time is equal to 0.05 seconds. Otherwise, step 1207 is skipped. Step 1208 determines if the Initial Switch Time is greater than the Final Switch Time. If this is true, then in step 1209 the values for the Initial and Final Switch Times are inverted. The routine concludes and, thus, concludes step 1113.

In step 1114, the Mobility (cm/hr) is determined in the same manner as was described for Case 2 (Fig. 9). In step 1115, the Run Distance is set equal to 4 cm. In step 1116, the Run Time is determined by multiplying the Calibration Factor by the Run Distance and dividing by the Mobility. The procedure concludes by returning.

while the invention is described in some detail with specific reference to a single preferred embodiment and certain alternatives, there is no intent to limit the invention to that particular embodiment or those specific alternatives. The true scope of the invention is defined

not by the foregoing description but by the following claims.

### Appendix

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5 The first column (X) is the Minimum DNA Size [Low] (Kilobases). The second column (Y) is the Switch Time (seconds). The third column (Z) is the Electrophoretic Mobility 10 (centimeters/hour). Data Z Y 15 . X 0.2 0.273 8.14 0.5 0.338 8.14 0.8 0.384 8.14 0.2 0.245 20 10.18 0.5 0.315 10.18 0.362 10.18 0.8 0.217 0.2 12.20 0.8 0.351 12.20 0.180 25 15.00 0.2 0.281 0.5 15.00 0.348 15.00 0.8 0.157 0.2 17.00 0.262 17.00 0.5 0.339 0.8 30 17.00 0.138 19.40 0.2 0.236 0.5 19.40 0.332 19.40 0.8 0.3 0.177 22.60 0.5 0.216 22.60 35

0.318

0.158

0.8

0.3

22.60

24.80

	24.80	0.5	0.191
	24.80	0.8	0.306
	29.90	0.3	0.128
	29.90	0.5	0.177
5	29.90	0.8	0.274
	33.50	0.3	0.144
	33.50	0.5	0.156
	33.50	0.8	0.245
	38.40	0.3	0.105
10	38.40	0.5	0.126
	38.40	0.8	0.210
	48.50	0.5	0.095
	48.50	0.8	0.146

15 The first column (X) is the Minimum DNA Size [Low] (Kilobases).

The second column (Y) is the Switch Time (seconds). The third column (2) is the Electrophoretic Mobility.

#### 20 Fitted Surface

X

	X	Y	z
	8.1400e+000	2 0000- 00-	
25	8.1400e+000	2.0000e-001 2.3000e-001	2.7129e-001
	8.1400e+000	2.6000e-001	2.8031e-001 2.8722e-001
	8.1400e+000	2.9000e-001	2.9268e-001
	8.1400e+000 8.1400e+000	3.2000e-001	2.9759e-001
30	8.1400e+000	3.5000e-001 3.8000e-001	3.0269e-001 3.0842e-001
	8.1400e+000	4.1000e-001	3.1486e-001
	8.1400e+000	4-4000e-001	3.2185e-001
	8.1400e+000 8.1400e+000	4.7000e-001	3.2911e-001
35	8.1400e+000	5.0000e-001 5.3000e-001	3.3611e-001 3.4220e-001
	8.1400e+000	5.6000e-001	3.4727e-001
	8.1400e+000	5.9000e-001	3.5142e-001

	8.1400e+000	6.2000e-001	3.5486e-001
	8.1400e+000	6.5000e-001	3.5794e-001
	8.1400e+000	6.8000e-001	3.6108e-001
	8.1400e+000	7.1000e-001	3.6467e-001
5	8.1400e+000	7.4000e-001	3.6903e-001
	8.1400e+000	7.7000e-001	3.7423e-001
	8.1400e+000	8.0000e-001	3.7958e-001
	1.0158e+001	2.0000e-001	2.4379e-001
10	1.0158e+001	2.3000e-001	2.5399e-001
	1.0158e+001	2.6000e-001	2.6236e-001
	1.0158e+001	2.9000e-001	2.6923e-001
	1.0158e+001	3.2000e-001	2.7539e-001
	1.0158e+001	3.5000e-001	2.8155e-001
15 ·	1.0158e+001	3.8000e-001	2.8814e-001
	1.0158e+001	4.1000e-001	2.9523e-001
	1.0158e+001	4.4000e-001	3.0264e-001
	1.0158e+001	4.7000e-001	3.1004e-001
	1.0158e+001	5.0000e-001	3.1720e-001
20	1.0158e+001	5.3000e-001	3.2401e-001
	1.0158e+001	5.6000e-001	3.3004e-001
	1.0158e+001	5.9000e-001	3.3519e-001
	1.0158e+001	6.2000e-001	3.3968e-001
	1.0158e+001	6.5000e-001	3.4383e-001
25	1.0158e+001	6.8000e-001	3.4795e-001
	1.0158e+001	7.1000e-001	3.5227e-001
	1.0158e+001	7.4000e-001	3.5690e-001
	1.0158e+001	7.7000e-001	3.6177e-001
	1.0158e+001	8.0000e-001	3.6691e-001
30			
	1.2176e+001	2.0000e-001	2.1715e-001
	1.2176e+001	2.3000e-001	
	1.2176e+001	2.6000 <b>e-</b> 001	
	1.2176e+001	2.9000e-001	
35	1.2176e+001	3.2000e-001	2.5435e-001
	1.2176e+001	3.5000e-001	
	1.2176e+001	3.8000e-001	2.6893e-001

	1.2176e+001	4 1000	
	1.2176e+001	4.1000e-001	2.7663e-001
	1.2176e+001	4.4000e-001	2.8453e-001
		4.7000e-001	2.9237e-001
5	1.2176e+001	5.0000e-001	2.9990e-001
-	1.2176e+001	5.3000e-001	3.0694e-001
	1.2176e+001	5.6000e-001	3.1339e-001
	1.2176e+001	5.9000e-001	3.1930e-001
	1.2176e+001	6.2000e-001	3.2481e-001
1.0	1.2176e+001	6.5000e-001	3.3013e-001
10	1.2176e+001	6.8000e-001	3.3540e-001
	1.2176e+001	7.1000e-001	3.4071e-001
	1.2176e+001	7.4000e-001	3.4605e-001
	1.2176e+001	7.7000e-001	3.5132e-001
	1.2176e+001	8.0000e-001	3.5688e-001
15		•	3000
	1.4194e+001	2.0000e-001	1 0304 055
	1.4194e+001	2.3000e-001	1.9194e-001
20	1.4194e+001	2.6000e-001	2.0479e-001
	1.4194e+001	2.9000e-001	2.1642e-001
	1.4194e+001	3.2000e-001	2.2635e-001
	1.4194e+001	3.5000e-001	2.3494e-001
	1.4194e+001	3.8000e-001	2.4292e-001
25	1.4194e+001	4.1000e-001	2.5084e-001
	1.4194e+001	4.4000e-001	2.5893e-001
	1.4194e+001	4.7000e-001	2.6725e-001
	1.4194e+001	5.0000e-001	2.7564e-001
	1.4194e+001	5.3000e-001	2.8362e-001
30	1.4194e+001	5.6000e-001	2.9064e-001
	1.4194e+001	5.9000e-001	2.9722e-001
	1.4194e+001	6.2000e-001	3.0374e-001
	1.4194e+001	6.5000 <b>e-</b> 001	3.1026e-001
	1.4194e+001	6.8000e-001	3.1678e-001
35	1.4194e+001	7.1000e-001	3.2333e-001
	1.4194e+001	7.4000e-001	3.2989e-001
	1.4194e+001	7.7000e-001	3.3643e-001
	<del>-</del>		3.4295e-001

	1.4194e+001	8.0000e-001	3.4960e-001
	1.6212e+001	2.0000e-001	1.6937e-001
	1.6212e+001	2.3000e-001	1.8355e-001
5	1.6212e+001	2.6000e-001	1.9689e-001
5	1.6212e+001	2.9000e-001	2.0824e-001
	1.6212e+001	3,2000e-001	2.1764e-001
	1.6212e+001	3.5000e-001	2.2594e-001
	1.6212e+001	3.8000e-001	2.3394e-001
10	1.6212e+001	4.1000e-001	2.4202e-001
10	1.6212e+001	4.4000e-001	2.5029e-001
	1.6212e+001	4.7000e-001	2.5870e-001
	1.6212e+001	5.0000e-001	2.6677e-001
	1.6212e+001	5.3000e-001	2.7406e-001
15 .	1.6212e+001	5.6000e-001	2.8112e-001
	1.6212e+001	5.9000e-001	2.8842e-001
	1.6212e+001	6.2000e-001	2.9598e-001
	1.6212e+001	6.5000e <del>-</del> 001	3.0371e-001
	1.6212e+001	6.8000e-001	3.1154e-001
20	1.6212e+001	7.1000e-001	3.1938e-001
	1.6212e+001	7.4000e-001	3.2717e-001
	1.6212e+001	7.7000e-001	3.3486e-001
	1.6212e+001	8.0000e-001	3.4240e-001
25			
	1.8230e+001	2.0000e-001	1.5044e-001
	1.8230e+001	2.3000e-001	1.6570e-001
	1.8230e+001	2.6000e-001	1.8059e-001
30	1.8230e+001	2.9000e-001	1.9314e-001
	1.8230e+001	3.2000e-001	2.0283e-001
	1.8230e+001	3.5000e-001	2.1081e-001
	1.8230e+001	3.8000e-001	2.1834e-001
	1.8230e+001	4.1000e-001	2.2594e-001
35	1.8230e+001	4.4000e-001	2.3368e-001
	1.8230e+001	4.7000e-001	2.4146e-001
	1.8230e+001	5.0000e-001	2.4922e-001
		· ·	

	1.8230e+001	5.3000e-001	2.5704e-001
	1.8230e+001	5.6000e-001	2.6504e-001
	1.8230e+001	5.9000e-001	2.7333e-001
	1.8230e+001	6.2000e-001	2.8195e-001
5	1.8230e+001	6.5000 <b>e-</b> 001	2.9082e-001
	1.8230e+001	6.8000 <b>e-</b> 001	2.9982e-001
	1.8230e+001	7.1000e-001	3.0883e-001
	1.8230e+001	7.4000e-001	3.1773e-001
	1.8230e+001	7.7000e-001	3.2641e-001
10	1.8230e+001	8.0000e-001	3.3472e-001
	2.0248e+001	2.0000e-001	1.3526e-001
	2.0248e+001	2.3000e-001	1.5140e-001
	2.0248e+001	2.6000e-001	1.6750e-001
15	2.0248e+001	2.9000e-001	1.8085e-001
	2.0248e+001	3.2000e-001	1.9028e-001
	2.0248e+001	3.5000e-001	1.9738e-001
	2.0248e+001	3.8000e-001	2.0406e-001
	2.0248e+001	4.1000e-001	2.1092e-001
20	2.0248e+001	4.4000e-001	2.1793e-001
	2.0248e+001	4.7000e-001	2.2507e-001
	2.0248e+001	5 <sub>0</sub> 0000e-001	2.3254e-001
	2.0248e+001	5.3000e-001	2.4068e-001
	2.0248e+001	5.6000e-001	2.4943e-001
25	2.0248e+001	5.9000e-001	2.5864e-001
	2.0248e+001	6.2000e-001	2.6823e-001
	2.0248e+001	6.5000e-001	2.7808e-001
	2.0248e+001	6.8000e-001	2.8806e-001
	2.0248e+001	7.1000e-001	2.9805e-001
30	2.0248e+001	7.4000e-001	3.0790e-001
	2.0248e+001	7.7000e-001	3.1746e-001
	2.0248e+001	8.0000e-001	3.2646e-001
35			
	2.2266e+001	2.0000e-001	1.2491e-001
	2.2266e+001	2.3000e-001	1.4088e-001

	2.2266e+001	2.6000e-001	1.5667e-001
	2.2266e+001	2.9000e-001	1.6970e-001
	2.2266e+001	3.2000e-001	1.7860e-001
	2.2266e+001	3.5000e-001	1.8493e-001
5	2.2266e+001	3.8000e-001	1.9091e-001
	2.2266e+001	4.1000e-001	1.9707e-001
	2.2266e+001	4.4000e-001	2.0335e-001
	2.2266e+001	4.7000e-001	2.0988e-001
	2.2266e+001	5.0000e-001	2.1711e-001
10	2.2266e+001	5.3000e-001	2.2534e-001
	2.2266e+001	5.6000e-001	2.3461e-001
	2.2266e+001	5.9000e-001	2.4457e-001
	2.2266e+001	6.2000e-001	2.5490e-001
	2.2266e+001	6.5000e-001	2.6547e-001
15	2.2266e+001	6.8000e-001	2.7617e-001
	2.2266e+001	7.1000e-001	2.8689e-001
	2.2266e+001	7.4000e-001	2.9749e-001
	2.2266e+001	7.7000e-001	3.0776e-001
	2.2266e+001	8.0000e-001	3.1732e-001
20			
	2.4284e+001	2.0000e-001	1.1872e-001
	2.4284e+001	2.3000e-001	1.3323e-001
	2.4284e+001	2.6000e-001	1.4694e-001
_	2.4284e+001	2.9000e-001	1.5821e-001
25	2.4284e+001	3.2000e-001	1.6653e-001
	2.4284e+001	3.5000e-001	1.7291e-001
	2.4284e+001	3.8000e-001	
	2.4284e+001	4.1000e-001	1.8449e-001
	2.4284e+001	4.4000e-001	1.9016e-001
30	2.4284e+001	4.7000e-001	1.9584e-001
	2.4284e+001	5.0000e-001	2.0242e-001
	2.4284e+001	5.3000e-001	2.1100e-001
	2.4284e+001	5.6000e-001	2.2091e-001
25	2.4284e+001	5.9000e-001	2.3134e-001
35		6.2000e-001	2.4208e-001
	2.4284e+001	6.5000e-001	2.5303e-001
	2.4284e+001	6.8000e-001	2.6411e-001

	2-4284e+001	7.1000e-001	2.7523e-001
	2.4284e+001	7.4000e-001	2.8625e-001
	2.4284e+001	7.7000e-001	2.9696e-001
	2.4284e+001	8.0000e-001	3.0691e-001
5			
		ě.	
	2.6302e+001	2.0000e-001	1.1480e-001
	2.6302e+001	2.3000e-001	1.2723e-001
10	2.6302e+001	2.6000e-001	1.3835e-001
	2.6302e+001	2.9000e-001	1.4760e-001
	2.6302e+001	3.2000e-001	1.5520e-001
	2.6302e+001	3.5000e-001	1.6171e-001
	2.6302e+001	3.8000e-001	1.6764e-001
15	2.6302e+001	4.1000e-001	1.7329e-001
	2.6302e+001	4.4000e-001	1.7881e-001
	2.6302e+001	4.7000e-001	1.8440e-001
	2.6302e+001	5.0000e-001	1.9087e-001
	2.6302e+001	5.3000e-001	1.9924e-001
20	2.6302e+001	5.6000e-001	2.0889e-001
	2.6302e+001	5.9000e-001	2.1913e-001
	2.6302e+001	6.2000e-001	2.2979e-001
	2.6302e+001	6.5000e-001	2.4074e-001
	2.6302e+001	6.8000e-001	2.5188e-001
25	2.6302e+001	7.1000e-001	2.6306e-001
	2.6302e+001	7.4000e-001	2.7413e-001
	2.6302e+001	7.7000e-001	2.8489e-001
	2.6302e+001	8.0000e-001	2.9497e-001
30	2.8320e+001	2.0000e-001	1.1212e-001
	2.8320e+001	2.3000e-001	1.2265e-001
	2.8320e+001	2.6000e-001	1.3138e-001
	2.8320e+001	2.9000e-001	1.3849e-001
	2.8320e+001	3.2000e-001	1.4532e-001
35	2.8320e+001	3.5000e-001	1.5190e-001
	2.8320e+001	3.8000e-001	1.5779e-001
	2.8320e+001	4.1000e-001	1.6332e-001

	2.8320e+001	4.4000e-001	1.6896e-001
	2.8320e+001	4.7000e-001	1.7516e-001
	2.8320e+001	5.0000e-001	1.8209e-001
	2.8320e+001	5.3000e-001	1.8965e-001
5	2.8320e+001	5.6000e-001	1.9819e-001
	2.8320e+001	5.9000e-001	2.0770e-001
	2.8320e+001	6.2000e-001	2.1791e-001
	2.8320e+001	6.5000e-001	2.2859e-001
	2.8320e+001	6.8000e-001	2.3952e-001
10	2.8320e+001	7.1000e-001	2.5052e-001
	2.8320e+001	7.4000e-001	2.6142e-001
	2.8320e+001	7.7000e-001	2.7204e-001
	2.8320e+001	8.0000e-001	2.8211e-001
15 ·			
	3.0338e+001	2.0000e-001	1.1022e-001
	3.0338e+001	2.3000e-001	1.1966e-001
	3.0338e+001	2.6000e-001	1.2721e-001
20	3.0338e+001	2.9000e-001	1.3283e-001
	3.0338e+001	3.2000e-001	1.3844e-001
	3.0338e+001	3.5000e-001	1.4414e-001
	3.0338e+001	3.8000e-001	1.4922e-001
	3.0338e+001	4.1000e-001	1.5416e-001
25	3.03 <b>38e</b> +001	4.4000e-001	1.5954e-001
	3.0338e+001	4.7000e-001	1.6574e-001
	3.0338e+001	5.0000e-001	1.7260e-001
	3.0338e+001	5.3000e-001	1.7974e-001
	3.0338e+001	5.6000e-001	1.8762e-001
30	3.0338e+001	5.9000e-001	1.9651e-001
	3.0338e+001	6.2000e-001	2.0623e-001
	3.0338e+001	6.5000e-001	2.1649e-001
	3.0338e+001	6.8000e-001	2.2707e-001
	3.0338e+001	7.1000e-001	2.3774e-001
35	3.0338e+001	7.4000e-001	2.4835e-001
	3.0338e+001	7.7000e-001	
	3.0338e+001	8.0000e-001	2.6883e-001
			•

	3.2356e+001	2.0000e-001	1.0830e-001
	3.2356e+001	2.3000e-001	1.1739e-001
	3.2356e+001	2.6000e-001	1.2552e-001
5	3.2356e+001	2.9000e-001	1.3183e-001
	3.2356e+001	3.2000e-001	1.3551e-001
	3.2356e+001	3.5000e-001	1.3826e-001
	3.2356e+001	3.8000e-001	1.4147e-001
	3.2356e+001	4.1000e-001	1.4534e-001
10	3.2356e+001	4.4000e-001	1.5000e-001
	3.2356e+001	4.7000e-001	1.5559e-001
	3.2356e+001	5.0000e-001	1.6197e-001
	3.2356e+001	5.3000e-001	1.6897e-001
	3.2356e+001	5.6000e-001	1.7670e-001
15	3.2356e+001	5.9000e-001	1.8526e-001
	3.2356e+001	6.2000e-001	1.9458e-001
	3.2356e+001	6.5000e-001	2.0443e-001
	3.2356e+001	6.8000e-001	2.1456e-001
	3.2356e+001	7.1000e-001	2.2478e-001
20	3.2356e+001	7.4000e-001	2.3490e-001
	3.2356e+001	7.7000e-001	2.4477e-001
	3.2356e+001	8.0000e-001	2.5437e-001
25			
	3.4374e+001	2.0000e-001	1.0519e-001
	3.4374e+001	2.3000e-001	1.1328e-001
	3.4374e+001	2.6000e-001	1.2088e-001
	3.4374e+001	2.9000e-001	1.2710e-001
30	3.4374e+001	3.2000e-001	1.3004e-001
	3.4374e+001	3.5000e-001	1.3144e-001
	3.4374 <del>e+</del> 001	3.8000e-001	1.3352e-001
	3.4374e+001	4.1000e-001	1.3648e-001
	3.4374e+001	4.4000e-001	1.403le-001
35	3.4374e+001	4.7000e-001	1.4517e-001
	3.4374e+001	5.0000e-001	1.5108e-001
	3.4374e+001	5.3000e-001	1.5789e-001

	3.4374e+001	5.6000e-001	1.6554e-001
	3.4374e+001	5.9000e-001	1.7396e-001
	3.4374e+001	6.2000e-001	1.8297e-001
	3.4374e+001	6.5000e-001	1.9241e-001
5	3.4374e+001	6.8000e-001	2.0207e-001
	3.4374e+001	7.1000e-001	2.1176e-001
	3.4374e+001	7.4000e-001	2.2134e-001
	3.4374e+001	7.7000e-001	2.3072e-001
	3.4374e+001	8.0000e-001	2.4001e-001
10			
	3.6392e+001	2.0000e-001	1.0050e-001
	3.6392e+001	2.3000e-001	1.0656e-001
	3.6392e+001	2.6000e-001	1.1172e-001
	3.6392e+001	2.9000e-001	1.1606e-001
15	3.6392e+001	3.2000e-001	1.1962e-001
	3.6392e+001	3.5000e-001	1.2243e-001
	3.6392e+001	3.8000e-001	1.2490e-001
	3.6392e+001	4.1000e-001	1.2749e-001
	3.6392e+001	4.4000e-001	1.3057e-001
20	3.6392e+001	4.7000e-001	1.3449e-001
	3.6392e+001	5.0000e-001	1.3975e-001
	3.6392e+001	5.3000e-001	1.4654e-001
	3.6392e+001	5.6000e-001	1.5436e-001
	3.6392e+001	5.9000e-001	1.6275e-001
25	3.6392e+001	6.2000e-001	1.7151e-001
	3.6392e+001	6.5000e-001	1.8054e-001
	3.6392e+001	6.8000e-001	1.8970e-001
	3.6392e+001	7.1000e-001	1.9884e-001
	3.6392e+001	7.4000e-001	2.0783e-001
30	3.6392e+001	7.7000 <b>e-</b> 001	2.1667e-001
	3.6392e+001	8.0000e-001	2.2561e-001
35	3.8410e+001	2.0000e-001	9.5101e-002
	3.8410e+001	2.3000e-001	9.9338 <b>e-</b> 002
	3.8410e+001	2.6000e-001	1.0269e-001
		•	

	2 0430		
	3.8410e+001	2.9000e-001	1.0589e-001
	3.8410e+001	3.2000e-001	1.0962e-001
	3.8410e+001	3.5000e-001	1.1328e-001
5	3.8410e+001	3.8000e-001	1.1622e-001
٥	3.8410e+001	4.1000e-001	1.1875e-001
	3.8410e+001	4.4000e-001	1.2134e-001
	3.8410e+001	4.7000e-001	1.2447e-001
	3.8410e+001	5.0000e-001	1.2905e-001
10	3.8410e+001	5.3000e-001	1.3577e-001
10	3.8410e+001	5.6000e-001	1.4370e-001
	3.8410e+001	5.9000e-001	1.5198e-001
	3.8410e+001	6.2000e-001	1.6040e-001
	3.8410e+001	6.5000e-001	1.6894e-001
3	3.8410e+001	6.8000e-001	1.7756e-001
15 .	3.8410e+001	7.1000e-001	1.8617e-001
	3.8410e+001	7.4000e-001	1.9465e-001
	3.8410e+001	7.7000e-001	2.0297e-001
	3.8410e+001	8.0000e-001	2.1149e-001
20	4.0428e+001	2.0000e-001	8.9854e-002
	4.0428e+001	2.3000e-001	9.3041e-002
	4.0428e+001	2.6000e-001	9.5698e-002
	4.0428e+001	2.9000e-001	9.8494e-002
	4.0428e+001	3.2000e-001	1.0183e-001
25	4.0428e+001	3.5000e-001	1.0521e-001
	4.0428e+001	3.8000e-001	1.0811e-001
	4.0428e+001	4.1000e-001	1.1067e-001
	4.0428e+001	4.4000e-001	1.1327e-001
	4.0428e+001	4.7000e-001	1.1638e-001
30	4.0428e+001	5.0000e-001	1.2077e-001
	4.0428e+001	5.3000e-001	1.2695e-001
	4.0428e+001	5.6000e-001	1.3429e-001
	4.0428e+001	5.9000e-001	1.4200e-001
	4.0428e+001	6.2000e-001	1.4982e-001
35	4.0428e+001	6.5000e-001	1.5772e-001
	4.0428e+001	6.8000e-001	1.6572e-001
	4.0428e+001	7.1000e-001	1.7381e-001

	4.0428e+001	7.4000e-001	1.8193e-001
	4.0428e+001	7.7000e-001	1.9007e-001
	4.0428e+001	8.0000e-001	1.9844e-001
			•
5			
	4.2446e+001	2.0000e-001	8.4994e-002
	4.2446e+001	2.3000e-001	8.7585e-002
	4.2446e+001	2.6000e-001	8.9906e-002
10	4.2446e+001	2.9000e-001	9.2371e-002
	4.2446e+001	3.2000e-001	9.5143e-002
	4.2446e+001	3.5000e-001	9.7984e-002
	4.2446e+001	3.8000e-001	1.0066e-001
	4.2446e+001	4.1000e-001	1.0330e-001
15	4.2446e+001	4.4000e-001	1.0619e-001
	4.2446e+001	0428e+001 7.7000e-001 1.0428e+001 8.0000e-001 1.0428e+001 8.0000e-001 1.0428e+001 2.0000e-001 1.02446e+001 2.9000e-001 1.02446e+001 3.5000e-001 1.02446e+001 3.8000e-001 1.02446e+001 4.1000e-001 1.02446e+001 5.0000e-001 1.02446e+001 5.0000e-001 1.02446e+001 5.0000e-001 1.02446e+001 5.0000e-001 1.02446e+001 5.0000e-001 1.02446e+001 5.0000e-001 1.02446e+001 6.5000e-001 1.02446e+001 6.5000e-001 1.02446e+001 7.1000e-001 1.02446e+001 7.000e-001 1.02446e+001 7.0	1.0968e-001
	4.2446e+001	5.0000e-001	1.1412e-001
	4.2446e+001	5.3000e-001	1.1969e-001
	4.2446e+001	5.6000e-001	1.2609e-001
20	4.2446e+001	5.9000e-001	1.3289e-001
	4.2446e+001	6.2000e-001	1.3984e-001
	4.2446e+001	6, 5000e-001	1.4691e-001
•	4.2446e+001	6.8000e-001	1.5415e-001
	4.2446e+001	7.1000e-001	1.6164e-001
25	4.2446e+001	7.4000e-001	1.6939e-001
	4.2446e+001	7.7000e-001	1.7738e-001
	4.2446e+001	8.0000e-001	1.8566e-001
	4.4464e+001	2.0000e-001	8.0402e-002
30	4.4464e+001	2.3000e-001	8.2518e-002
	4.4464e+001	2.6000e-001	8.4492e-002
	4.4464e+001	2.9000e-001	8.6564e-002
	4.4464e+001	3.2000e-001	8.8827e-002
	4.4464e+001	3.5000e-001	9.1218e-002
35	4.4464e+001	3.8000e-001	9.3707e-002
	4.4464e+001	4.1000e-001	9.6422e-002
	4.4464e+001	4.4000e-001	9.957 <b>8e-</b> 002

	4.4464e+001	4.7000e-001	1.0335e-001
	4.4464e+001	5.0000e-001	1.0781e-001
	4.4464e+001	5.3000e-001	1.1293e-001
	4.4464e+001	5.6000e-001	1.1852e-001
5	4.4464e+001	5.9000e-001	1.2438e-001
	4.4464e+001	6.2000e-001	1.3036e-001
	4.4464e+001	6.5000e-001	1.3648e-001
	4.4464e+001	6.8000e-001	1.4286e-001
	4.4464e+001	7.1000e-001	1.4962e-001
10	4.4464e+001	7.4000e-001	1.5684e-001
	4.4464e+001	7.7000e-001	1.6452e-001
	4.4464e+001	8.0000e-001	1.7259e-001
15			
		2.0000e-001	7.5959e-002
		2.3000e-001	7.7601e-002
	4.6482e+001	2.6000e-001	7.9176e-002
	4.6482e+001	2.9000e-001	8.0843e-002
20	4.6482e+001	3.2000e-001	8.2690e-002
	4.6482e+001	3.5000e-001	8.4756e-002
	4.6482e+001	3.8000e-001	8.7101e-002
	4.6482e+001	4.1000e-001	8.9849e-002
	4.6482e+001	4.4000e-001	9.3127e-002
25	4.6482e+001	4.7000e-001	9.6985e-002
	4.6482e+001	5.0000e-001	1.0136e-001
	4.6482e+001	5.3000e-001	1.0612e-001
	4.6482e+001	5.6000e-001	1.1109e-001
	4.6482e+001	5.9000e-001	1.1613e-001
30	4.6482e+001	6.2000e-001	1.2120e-001
	4.6482e+001	6.5000e-001	1.2639e-001
	4.6482e+001	6.8000e-001	1.3188e-001
	4.6482e+001	7.1000e-001	1.3785e-001
_	4.6482e+001	7.4000e-001	1.4442e-001
35	4.6482e+001	7.7000e-001	1.5161e-001
	4.6482e+001	8.0000e-001	1.5934e-001

	4.8500e+001	2.0000e-001	7.1639e-002	
	4.8500e+001	2.3000e-001	7.2801e-002	
	4.8500e+001	2.6000e-001	7.3961e-002	
	4.8500e+001	2.9000e-001	7.5246e-002	
5	4.8500e+001	3.2000e-001	7.67 <b>55e-</b> 002	
	4.8500e+001	3.5000e-001	7.8571e-002	
	4.8500e+001	3.8000e-001	8.0784e-002	
	4.8500e+001	4.1000e-001	8.3490e-002	
	4.8500e+001	4.4000e-001	8.6750e-002	
10	4.8500e+001	4.7000e-001	9.0536e-002	
	4.8500e+001	5.0000e-001	9.4723e-002	
	4.8500e+001	5.3000e-001	9.9123e-002	
	4.8500e+001	5.6000e-001	1.0356e-001	
•	4.8500e+001	5.9000e-001	1.0791e-001	
15	4.8500e+001	6.2000e-001	1.1220e-001	
	4.8500e+001	6.5000e-001	1.1657e-001	
	4.8500e+001	6.8000e-001	1.2123e-001	
	4.8500e+001	7.1000e-001	1.2643e-001	
	4.8500e+001	7.4000e-001	1.3232e-001	
20	4.8500e+001	7.7000e-001	1.3894e-001	
	4.8500e+001	8.0000e-001	1.4621e-001	

Table 2.

5	The first column (X) is the Minimum DNA Size [Low] (Kilobases).
	The second column (Y) is the Switch Time (seconds). The third column (Z) is the Electrophoretic Mobility.

## 10 Data

	X	Y	2
	48.5	30	0.3056
15	48.5	75	0.3150
	48.5	90	0.3200
	97.0	5	0.1540
	97.0	10	0.2100
	97.0	15	0.2719
20	97.0	30	0.2819
	97.0	45	0.2790
	97.0	70	0.2950
	97.0	90	0.3067
	97.0	105	0.3155
25	145.5	15	0.2256
	145.5	30	0.2588
	145.5	45	0.2605
	145.5	60	0.2857
	145.5	75	0.2918
30	145.5	90	0.2942
	145.5	105	0.3060
	194.0	15	0.1677
	194.0	30	0.2356
	194.0	45	0.2437
35	194.0	60	0.2714
	194.0	75	0.2767
	194.0	90	0.2834

	194.0	105	0.2960
	242.5	15	0.1157
	242.5	20	0.1600
	242.5	30	0.2119
5	242.5	45	0.2260
	242.5	60	0.2571
	242.5	75	0.2666
	242.5	90	0.2726
	242.5	105	0.2860
10	291.0	15	0.0925
	291.0	30	0.1863
	291.0	45	0.2084
	291.0	60	0.2452
	291.0	75	0.2541
15	291.0	90	0.2618
	291.0	105	0.2759
	339.5	15	0.0690
	339.5	30	0.1528
	339.5	45	0.1907
20	339.5	60	0.2310
	339.5	75	0.2440
	339.5	90	0.2510
	339.5	105	0.2660
	388.0	30	0.1183
25	388.0	45	0.1714
	388.0	60	0.2190
	388.0	75	0.2314
	388.0	90	0.2414
	388.0	105	0.2583
30	436.5	30	0.0932
	436.5	45	0.1479
	436.5	60	0.2048
	436.5	75	0.2214
	436.5	90	0.2317
35	436.5	105	0.2508
	485.0	30	0.0789
	485.0	45	0.1176

	485.0	60	0.1905
	485.0	75	0.2098
	485.0	90	0.2215
	485.0	105	0.2408
5	533.5	40	0.0750
	533.5	45	0.0924
	533.5	60	0.1762
	533.5	75	0.1987
	533.5	90	0.2101
10	533.5	105	0.2333
	582.0	40	0.0550
	582.0	45	0.0748
	582.0	60	0.1595
	582.0	75	0.1887
15	582.0	90	0.2011
	582.0	105	0.2257
	630.5	40	0.0400
	630.5	45	0.0639
	630.5	60	0.1333
20	630.5	75	0.1786
	630.5	90	0.1931
	630.5	105	0.2157
	679.0	60	0.1095
	679.0	75	0.1660
25	679.0	90	0.1863
	679.0	105	0.2057
	727.5	60	0.0881
	727.5	75	0.1459
	727.5	90	0.1766
30	776.0	60	0.0714
	776.0	75	0.1233
	776.0	90	0.1647
	832.0	60	0.0620
	832.0	75	0.0950
35	832.0	90	0.1425
	832.0	105	0.1800
	832.0	120	0.1837

915.0	75	0.0705
915.0	90	0.1070
915.0	105	0.1650
915.0	120	0.1782

5

The first column (X) is the Minimum DNA Size [Low] (Kilobases).

The second column (Y) is the Switch Time (seconds). The third column (2) is the Electrophoretic Mobility.

10

## Fitted Surface

	x	Y	Z
15 .	4.8500e+001	5.0000e+000	2.1572e-001
	4.8500e+001	1.0750e+001	2.4990e-001
	4.8500e+001	1.6500e+001	2.7524e-001
	4.8500e+001	2.2250e+001	2.8992e-001
	4.8500e+001	2.8000e+001	3.0062e-001
20	4.8500e+001	3.3750e+001	3.0761e-001
_	4.8500e+001	3.9500e+001	3.0930e-001
	4.8500e+001	4.5250e+001	3.0986e-001
	4.8500e+001	5.1000e+001	3.1237e-001
	4.8500e+001	5.6750 <b>e</b> +001	3.1459e-001
2.5	4.8500e+001	6.2500e+001	3.1518e-001
	4.8500e+001	6.8250e+001	3.1519e-001
	4.8500e+001	7.4000e+001	3.1607e-001
	4.8500e+001	7.9750e+001	3.1763e-001
	4.8500e+001	8.5500e+001	3.1890e-001
30	4.8500e+001	9.1250e+001	3.1923e-001
	4.8500e+001	9.7000e+001	3.1973e-001
	4.8500e+001	1.0275e+002	3.2037e-001
	4.8500e+001	1.0850e+002	3.1918e-001
	4.8500e+001	1.1425e+002	3.1534e-001
35	4.8500e+001	1.2000e+002	3.0926e-001

	1.3515e+002	7.9750e+001	2.9725 <b>e-</b> 001
	1.3515e+002	7.4000e+001	2.9471e-001
35	1.3515e+002	6.8250e+001	2.9233e-001
35	1.3515e+002	6.2500e+001	2.8994e-001
	1.3515e+002	5.6750e+001	2.8586e-001
	1.3515e+002	5.1000e+001	2.7968e-001
	1.3515e+002	4.5250e+001	2.7284e-001
30	1.3515e+002	3.9500e+001	2.6808e-001
3.0	1.3515e+002	3.3750e+001	2.6321e-001
	1.3515e+002	2.8000e+001	2.5327e-001
	1.3515e+002	2.2250e+001	2.3880e-001
	1.3515e+002	1.6500e+001	2.2009e-001
25	1.3515e+002	1.0750e+001	1.9044e-001
25	1.3515e+002	5.0000e+000	1.5246e-001
	9.1825e+001	1.2000e+002	3.0448e-001
	9.1825e+001	1.1425e+002	3.0986e-001
20	9.1825e+001	1.0850e+002	3.1302e-001
2.0	9.1825e+001	1.0275e+002	3.1344e-001
	9.1825e+001	9.7000e+001	3.1170e-001
	9.1825e+001	9.1250e+001	3.0997e-001
	9.1825e+001	8.5500e+001	3.0899e-001
15 .	9.1825e+001	7.9750e+001	3.0742e-001
	9.1825e+001	7.4000e+001	3.0507e-001
	9.1825e+001	6.8250e+001	3.0310e-001
	9.1825e+001	6.2500e+001	3.0209e-001
	9.1825e+001	5.6750e+001	2.9984e-001
10	9.1825e+001	5.1000e+001	2.9533e-001
	9.1825e+001	4.5250e+001	2.9026e-001
	9.1825e+001	3.9500e+001	2.8790e-001
	9.1825e+001	3.3750e+001	2.8517e-001
	9.1825e+001	2.8000e+001	2.7712e-001
5	9.1825e+001	2.2250e+001	2.6541e-001
_	9.1825e+001	1.6500e+001	2.4958e-001
	9.1825e+001	1.0750e+001	2.2043e-001
	9.1825e+001	5.0000e+000	1.8147e-001

	1.3515e+002	8.5500e+001	2.9 <b>905e-</b> 001
	1.3515e+002	9.1250e+001	3.0058e-001
	1.3515e+002	9.7000e+001	3.0308e-001
	1.3515e+002	1.0275e+002	3.05 <b>33e</b> -001
5	1.3515e+002	1.0850e+002	3.0552e-001
	1.3515e+002	1.1425e+002	3.0318e-001
	1.3515e+002	1.2000e+002	2.9867e-001
10			
	1.7848e+002	5.0000e+000	1.2463e-001
	1.7848e+002	1.0750e+001	1.5894e-001
	1.7848e+002	1.6500e+001	1.8783e-001
	1.7848e+002	2.2250e+001	2.1054e-001
15	1.7848e+002	2.8000e+001	2.2902e-001
	1.7848e+002	3.3750e+001	2.4163e-001
	1.7848e+002	3.9500e+001	2.4907e-001
	1.7848e+002	4.5250e+001	2.5618e-001
	1.7848e+002	5.1000e+001	2.6458e-001
20	1.7848e+002	5.6750e+001	2.7215e-001
	1.7848e+002	6.2500e+001	2.7777e-001
	1.7848e+002	6.8250e+001	2.8143e-001
	1.7848e+002	7.4000e+001	2.8420e-001
•	1.7848e+002	7.9750e+001	2.8702e-001
25	1.7848e+002	8.5500e+001	2.8921e-001
	1.7848e+002	9.1250e+001	2.9123e-001
	1.7848e+002	9.7000e+001	2.9419e-001
	1.7848e+002	1.0275e+002	2.9677e-001
	1.7848e+002	1.0850e+002	2.9744e-001
30	1.7848e+002	1.1425e+002	2.9582e-001
	1.7848e+002	1.2000e+002	2.9215e-001
	2.2180e+002	5.0000e+000	9.3584e-002
	2.2180e+002	1.0750e+001	1.2529e-001
35	2.2180e+002	1.6500e+001	1.5495e-001
	2.2180e+002		
	2.2180e+002	2.8000e+001	2.0457e-001

	2.2180e+002	3.3750e+001	2.2010e-001
	2.2180e+002	3.9500e+001	2.3015e-001
	2.2180e+002	4.5250e+001	2.3953e-001
	2.2180e+002	5.1000e+001	2.4955e-001
5	2.2180e+002	5.6750e+001	2.5853e-001
	2.2180e+002	6.2500e+001	2.6543e-001
	2.2180e+002	6.8250e+001	2.7011e-001
	2.2180e+002	7.4000e+001	2.7355e-001
	2.2180e+002	7.9750e+001	2.7685e-001
10	2.2180e+002	8.5500e+001	2.7947e-001
	2.2180e+002	9.1250e+001	2.8193e-001
	2.2180e+002	9.7000e+001	2.8525e-001
	2.2180e+002	1.0275e+002	2.8811e-001
_	2.2180e+002	1.0850e+002	2.8917e-001
15	2.2180e+002	1.1425e+002	2.8816e-001
	2.2180e+002	1.2000e+002	2.8526e-001
20	2.6512 <b>e</b> +002	5.0000e+000	
	2.6512e+002		6.2018e-002
	2.6512e+002	1.0750e+001 1.6500e+001	9.3450e-002
	2.6512e+002	2.2250e+001	1.2447e-001
	2.6512e+002	2.8000e+001	1.5449e-001
25 .	2.6512e+002	3.3750e+001	1.8017e-001
	2.6512e+002	3.9500e+001	1.9835e-001
	2.6512e+002	4.5250e+001	2.1100e-001
	2.6512e+002	5.1000e+001	2.2266e-001
	2.6512e+002	5.6750e+001	2.3443e-001 2.4496e-001
30	2.6512e+002	6.2500e+001	2.5313e-001
	2.6512e+002	6.8250e+001	2.5872e-001
	2.6512e+002	7.4000e+001	2.6291e-001
	2.6512e+002	7.9750e+001	2.6678e-001
	2.6512e+002	8.5500e+001	2.6980e-001
35	2.6512e+002	9.1250e+001	2.7268e-001
	2.6512e+002	9.7000e+001	2.7636e-001
	2.6512 <del>e+</del> 002	1.0275e+002	2.7948e-001

	2.6512 <b>e</b> +002	1.0850e+002	2.8090e-001
	2.6512e+002	1.1425e+002	2.8045e-001
	2.6512e+002	1.2000e+002	2.7824e-001
			•
5	3.0845e+002	5.0000e+000	3.2160e-002
	3.0845e+002	1.0750e+001	6.4833e-002
	3.0845e+002	1.6500e+001	9.6914e-002
	3.0845e+002	2.2250e+001	1.2816e-001
	3.0845e+002	2.8000e+001	1.5563e-001
10	3.0845e+002	3.3750e+001	1.7604e-001
	3.0845e+002	3.9500e+001	1.9140e-001
	3.0845e+002	4.5250e+001	2.0546e-001
	3.0845e+002	5.1000e+001	2.1909e-001
	3.0845e+002	5.6750e+001	2.3130e-001
15	3.0845e+002	6.2500e+001	2.4079e-001
	3.0845e+002	6.8250e+001	2.4729e-001
	3.0845e+002	7.4000e+001	2.5228e-001
	3.0845e+002	7.9750e+001	2.5676e-001
	3.0845e+002	8.5500e+001	2.6022e-001
20	3.0845e+002	9.1250e+001	2.6352e-001
	3.0845e+002	9.7000e+001	2.6760e-001
	3.0845e+002	1.0275e+002	2.7103e-001
	3.0845e+002	1.0850e+002	2.7281e-001
	3.0845e+002	1.1425e+002	2.7288e-001
25	3.0845e+002	1.2000e+002	2.7128e-001
			3.8103e-003
	3.5178e+002	5.0000e+000	3.8103e-003
30	3.5178e+002	1.0750e+001	7.0912e-002
	3.5178e+002	1.6500e+001	•
	3.5178e+002	2.2250e+001	1.0275e-001
	3.5178e+002	2.8000e+001	1.3090e-001
	3.5178e+002	3.3750e+001	1.5313e-001
35	3.5178e+002	3.9500e+001	1.7123e-001
	3.5178e+002	4.5250e+001	1.8771e-001
	3.5178e+002	5.1000e+001	2.0332e-001

	3.5178e+002	5.6750e+001	2.1738e-001
	3.5178e+002	6.2500e+001	2.2831e-001
	3.5178e+002	6.8250e+001	2.3579 <b>e</b> -001
	3.5178e+002	7.4000e+001	2.4164e-001
5	3.5178e+002	7.9750e+001	2.4680e-001
	3.5178e+002	8.5500e+001	2.5074e-001
	3.5178 <b>e</b> +002	9.1250e+001	2.5451e-001
	3.5178e+002	9.7000e+001	2.5907e-001
	3.5178e+002	1.0275e+002	2.6291e-001
10	3.5178e+002	1.0850e+002	2.6510e-001
	3.5178e+002	1.1425e+002	2.6562 <b>e-</b> 001
	3.5178e+002	1.2000e+002	2.6453e-001
	3.9510e+002	5.0000e+000	0.0000e+000
15	3.9510e+002	1.0750e+001	1.2028e-002
	3.9510e+002	1.6500e+001	4.6202e-002
	3.9510e+002	2.2250e+001	7.8499e-002
	3.9510 <b>e</b> +002	2.8000e+001	1.0673e-001
	3.9510e+002	3.3750e+001	1.3022e-001
20	3.9510e+002	3.9500e+001	1.5045e-001
	3.9510e+002	4.5250e+001	1.6909e-001
	3.9510e+002	5.1000e+001	1.8689e-001
	3.9510e+002	5.6750e+001	2.0309e-001
	3.9510e+002	6.2500e+001	2.1562e-001
25	3.9510e+002	6.8250e+001	2.2417e-001
	3.9510e+002	7.4000e+001	2.3094e-001
	3.9510e+002	7.9750e+001	2.3684e-001
	3.9510e+002	8.5500e+001	2.4135e-001
	3.9510e+002	9.1250e+001	2.4567e-001
30	3.9510e+002	9.7000e+001	2.5075e-001
	3.9510e+002	1.0275e+002	2.5513e-001
	3.9510e+002	1.0850e+002	2.5778e-001
	3.9510e+002	1.1425e+002	2.5868e-001
	3.9510e+002	1.2000e+002	2.5799e-001
35			

	4.3843e+002	5.0000e+000	0.0000e+000
	4.3843e+002	1.0750e+001	0.000 <b>0e+0</b> 00
	4.3843e+002	1.6500e+001	2.2665e-002
	4.3843e+002	2.2250e+001	5.5437e-002
5	4.3843e+002	2.8000e+001	8.3931e-002
	4.3843e+002	3.3750e+001	1.0788 <b>e-</b> 001
	4.3843e+002	3.9500e+001	1.2905e-001
	4.3843e+002	4.5250e+001	1.4937e-001
	4.3843e+002	5.1000e+001	1.6958e-001
10	4.3843e+002	5.6750e+001	1.8823e-001
	4.3843e+002	6.2500e+001	2.0255e-001
	4.3843e+002	6.8250e+001	2.1237e-001
	4.3843e+002	7.4000 <b>e</b> +001	2.2023e-001
	4.3843e+002	7.9750e+001	2.26 <b>89e-</b> 001
15	4.3843e+002	8.5500e+001	2.3197e-001
	4.3843e+002	9.1250e+001	2.3687e-001
	4.3843e+002	9.7000e+001	2.4249e-001
	4.3843e+002	1.0275e+002	2.4741e-001
	4.3843e+002	1.0850e+002	2.5055e-001
20	4.3843e+002	1.1425e+002	2.5186e-001
	4.3843e+002	1.2000e+002	2.5156e-001
	4.8175e+002	5.0000e+000	0.0000e+000
	4.8175e+002	1.0750e+001	0.0000e+000
25	4.8175e+002	1.6500e+001	0.0000e+000
	4.8175 <b>e</b> +002	2.2250e+001	3.2418e-002
	4.8175e+002	2.8000e+001	6.1666e-002
	4.8175e+002		8.5536e-002
	4.8175e+002	3.9500e+001	
30	4.8175e+002	4.5250e+001	1.2873e-001
	4.8175e+002	5.1000e+001	1.5156e-001
	4.8175e+002	5.6750e+001	1.7284e-001
	4.8175e+002	6.2500e+001	
	4.8175e+002	6.8250e+001	
35	4.8175e+002	7.4000e+001	
	4.8175e+002	7.9750e+001	
	4.8175 <del>e+</del> 002	8.5500e+001	2.2254e-001

	4.8175 <b>e</b> +002	9.1250e+001	2.2801e-001
	4.8175e+002	9.7000e+001	2.3421e-001
	4.8175e+002	1.0275e+002	2.3962e-001
	4.8175 <b>e</b> +002	1.0850e+002	2.4323e-001
5	4.8175e+002	1.1425e+002	2.4503e-001
	4.8175e+002	1.2000e+002	2.4515e-001
		V	
10	5.2507e+002	5.0000e+000	0.0000e+000
	5.2507 <b>e</b> +002	1.0750e+001	0.0000e+000
	5.2507 <b>e</b> +002	1.6500e+001	0.0000e+000
	5.2507 <b>e+002</b>	2.2250e+001	7.4310e-003
	5.2507e+002	2.8000e+001	3.6980e-002
15	5.2507e+002	3.3750e+001	6.1612e-002
	5.2507e+002	3.9500e+001	8.4377e-002
	5.2507e+002	4.5250e+001	1.0821e-001
	5.2507e+002	5.1000e+001	1.3337e-001
	5.2507e+002	5.6750e+001	1.5700e-001
20	5.2507e+002	6.2500e+001	1.7513e-001
	5.2507e+002	6.8250e+001	1.8788e-001
	5.2507e+002	7.4000e+001	1.9832e-001
	5.2507e+002	7.9750e+001	2.0676e-001
	5.2507 <b>e+002</b>	8.5500e+001	2.1305e-001
25	5.2507e+002	9.1250e+001	2.1912e-001
	5.2507e+002	9.7000e+001	2.2595e-001
	5.2507e+002		2.3193e-001
	5.2507 <b>e+00</b> 2	1.0850e+002	2.3603e-001
	5.2507 <b>e+00</b> 2	1.1425e+002	2.3822e-001
30	5.2507 <b>e</b> +002	1.2000e+002	2.3870e-001
	5.6840e+002	5.0000e+000	0.0000e+000
	5.68 <b>40e+0</b> 02	1.0750e+001	0.0000e+000
	5.6 <b>840e+002</b>	1.6500e+001	0.0000e+000
35	5.68 <b>40e+</b> 002	2.2250e+001	0.0000e+000
	5.6840e+002	2.8000 <del>e+</del> 001	1.0962e-002
	5.6840 <del>e+</del> 002	3.3750e+001	3.7679e-002

	6.1172e+002	1.0850e+002	2.2121e-001
	6.1172e+002	1,0275e+002	2.1629e-001
35	6.1172e+002	9.7000 <b>e</b> +001	2.0952e-001
	6.1172e+002	9.1250e+001	2.0173e-001
	6.1172e+002	8.5500e+001	1.9414e-001
	6.1172e+002	7.9750e+001	1.8611e-001
	6.1172e+002	7.4000e+001	1.7534e-001
30	6.1172e+002	6.8 <b>250e</b> +001	1.6123e-001
	6.1172e+002	6.2500e+001	1.4452e-001
	6.1172e+002	5.6750e+001	1.2337e-001
	6.1172e+002	5.1000e+001	9.7704e-002
	6.1172e+002	4.5250e+001	7.0605e-002
25	6.1172e+002	3.9500e+001	4.3372e-002
	6.1172e+002	3.3750e+001	1.5390e-002
	6.1172e+002	2.8000e+001	0.0000e+000
	6.1172e+002	2.2250e+001	0.0000 <b>e</b> +000
	6.1172e+002	1.6500e+001	0.0000e+000
20	6.1172e+002	1.0750e+001	0.0000e+000
-	6.1172 <b>e</b> +002	5.0000e+000	0.0000e+000
15	5.6840e+002	1.2000e+002	2.3212e-001
	5.6840e+002	1.1425e+002	2.3132e-001
	5.6840e+002	1.0850e+002	2.2879e-001
	5.6840e+002	1.0275e+002	2.2424e-001
	5.6840e+002	9.7000e+001	2.1775e-001
10	5.6840e+002	9.1250e+001	2.1037e-001
	5.6840e+002	8.5500e+001	2.0361e-001
	5.6840e+002	7.9750e+001	1.9658e-001
	5.6840e+002	7.4000e+001	1.8707e-001
	5.6840e+002	6.8250e+001	1.7495e-001
5	5.6840e+002	6.2500e+001	1.6039e-001
	5.6840e+002	5.67 <b>50e</b> +001	1.40 <b>59e-</b> 001
	5.6840e+002	5.1000e+001	1.1544e-001
	5.6840e+002	4.5250e+001	8.8843e-002
	5.6840e+002	3.9500e+001	6.30 <b>75e-</b> 002

	6.1172 <b>e</b> +002	1.1425e+002	2.2412e-001
	6.1172 <b>e</b> +002	1.2000e+002	2.2524e-001
		•	
5	6.5505e+002	5.0000e+000	0.0000e+000
5	6.5505 <b>e</b> +002	1.0750e+001	0.0000e+000
	6.5505e+002	1.6500e+001	0.0000e+000
	6.5505 <b>e</b> +002	2.2250e+001	0.0000e+000
	6.5505e+002	2.8000e+001	0.0000e+000
	6.5505e+002	3.3750e+001	0.0000e+000
10	6.5505e+002	3.9500e+001	2.3705e-002
	6.5505e+002	4.5250e+001	5.2252e-002
	6.5505e+002	5.1000e+001	7.9827e-002
	6.5505e+002	5.6750e+001	1.0562e-001
	6.5505e+002	6.2500e+001	1.2780e-001
15 .	6.5505 <b>e</b> +002	6.8250e+001	1.4659e-001
	6.5505e+002	7.4000e+001	1.6269e-001
	6.5505e+002	7.9750e+001	1.7493e-001
	6.5505e+002	8.5500e+001	1.8432e-001
	6.5505e+002	9.1250e+001	1.9297e-001
20	6.5505e+002	9.7000e+001	2.0112e-001
	6.5505e+002	1.0275e+002	2.0805e-001
	6.5505e+002	1.0850e+002	2.1328e-001
	6.5505e+002	1.1425e+002	2.1655e-001
	6.5505e+002	1.2000e+002	2.1797e-001
25			3,2,0,7,2,001
	<b>6.000</b>		
	6.9838e+002	5.0000e+000	0.0000e+000
30	6.9838e+002	1.0750e+001	0.0000e+000
30	6.9838e+002	1.6500e+001	0.0000e+000
	6.9838e+002	2.2250e+001	0.0000e+000
	6.9838e+002	2.8000e+001	0.0000e+000
	6.983 <b>8e</b> +002	3.3750e+001	0.0000e+000
	6.9838 <b>e</b> +002	3.9500 <b>e</b> +001	3.6517 <b>e-</b> 003
35	6.9838 <b>e</b> +002	4.5250e+001	3.3617e-002
	6.9838 <b>e</b> +002	5.1000e+001	6.2015e-002
	6.9838e+002	5.6750e+001	8.8006e-002

	6.9838e+002	6.2500e+001	1.1087e-001
	6.9838e+002	6.8250e+001	1.3108e-001
	6.9838e+002	7.4000e+001	1.4867e-001
	6.9838e+002	7.9750e+001	1.6251e-001
5	6.9838e+002	8.5500e+001	1.7365e-001
3	6.9838e+002	9.1250e+001	1.8366e-001
	6.9838e+002	9.7000e+001	1.9238e-001
	6.9838e+002	1.0275e+002	1.9963e-001
	6.9838e+002	1.0850e+002	2.0517e-001
10	6.9838e+002	1.1425e+002	2.0867e-001
	6.9838e+002	1.2000e+002	2.1027e-001
	7.4170e+002	5.0000e+000	0.0000e+000
	7.4170e+002	1.0750e+001	0.0000e+000
15	7.4170e+002	1.6500e+001	0.0000e+000
	7.4170e+002	2.2250e+001	0.0000e+000
	7.4170e+002	2.8000e+001	0.0000e+000
	7.4170e+002	3.3750e+001	0.0000e+000
	7.4170e+002	3.9500e+001	0.0000e+000
20	7.4170e+002	4.5250e+001	1.5670e-002
	7.4170e+002	5.1000e+001	4.4955e-002
	7.4170e+002	5.6750e+001	7.1240e-002
	7.4170e+002	6.2500e+001	9.4380e-002
	7.41 <b>70e</b> +002	6.8250e+001	1.1497e-001
25	7.4170e+002	7.4000e+001	1.3320e-001
	7.4170e+002	7.9750e+001	1.4860e-001
	7.4170e+002	8.5500e+001	1.6173e-001
	7.4170e+002	9.1250e+001	1.7332e-001
	7.4170e+002	9.7000e+001	1.8308e-001
30	7.4170e+002	1.0275e+002	1.9107e-001
	7.4170e+002	1.0850e+002	1.9703e-001
	7.4170e+002	1.1425e+002	2.0058e-001
	7.4170e+002	1.2000e+002	2.0221e-001

	7.8502e+002	5.0000e+000	0.0000e+000
	7.8502e+002	1.0750e+001	0.0000e+000
	7.8502e+002	1.6500e+001	0.0000e+000
	7.8502e+002	2.2250e+001	0.0000e+000
5	7.8502e+002	2.8000e+001	0.0000e+000
	7.8502e+002	3.3750e+001	0.0000e+000
	7.8502e+002	3.9500e+001	0.0000e+000
	7.8502e+002	4.5250e+001	0.0000e+000
	7.8502e+002	5.1000e+001	2.9040e-002
10	7.8502e+002	5.6750e+001	5.6001e-002
	7.8502e+002	6.2500e+001	7.8938e-002
	7.8502e+002	6.8250e+001	9.8700e-002
	7.8502e+002	7.4000e+001	1.1671e-001
	7.8502e+002	7.9750e+001	1.3337e-001
15	7.8502 <b>e</b> +002	8.5500e+001	1.4833e-001
	7.8502e+002	9.1250e+001	1.6158e-001
	7.8502e+002	9.7000e+001	1.7297e-001
	7.8502e+002	1.0275e+002	1.8245e-001
20	7.8502e+002	1.0850e+002	1.8911e-001
20	7.8502e+002	1.1425e+002	1.9261e-001
	7.8502e+002	1.2000e+002	1.942le-001
	0.000	₩.	
	8.2835e+002	5.0000e+000	0.0000e+000
25	8.2835e+002	1.0750e+001	0.0000e+000
	8.2835e+002	1.6500e+001	0.0000e+000
	8.2835e+002	2.2250e+001	0.0000e+000
	8.2835 <b>e</b> +002	2.8000e+001	0.0000e+000
	8.2835e+002	3.3750e+001	0.0000e+000
30	8.2835e+002	3.9500e+001	0.0000e+000
30	8.2835e+002	4.5250e+001	0.0000e+000
	8.2835e+002	5.1000e+001	1.3539e-002
	8.2835e+002	5.6750e+001	4.1689e-002
	8.2835e+002	6.2500e+001	6.4196e-002
35	8.2835e+002	6.8250e+001	8.2402e-002
JJ	8.2835e+002	7.4000 <del>e+</del> 001	9.9799e-002
	8.2835e+002	7.9750e+001	1.1737e-001
	8.2835e+002	8.5500 <del>e+</del> 001	1.3360e-001

	8.2835e+002	9.1250e+001	1.4829e-001	
	8.2835e+002	9.7000e+001	1.6186e-001	
	8.2835e+002	1.0275e+002	1.7352e-001	
	8.2835e+002	1.0850e+002	1.8144e-001	
5	8.2835e+002	1.1425e+002	1.8542e-001	
	8.2835e+002	1.2000e+002	1.8746e-001	
10	8.7168e+002	5.0000e+000	0.0000e+000	
	8.7168e+002	1.0750e+001	0.0000e+000	
	8.7168e+002	1.6500e+001	0.0000e+000	
	8.7168e+002	2.2250e+001	0.0000e+000	
	8.7168e+002	2.8000e+001	0.0000e+000	
15	8.7168e+002	3.3750e+001	0.0000e+000	
	8.71 <b>68e</b> +002	3.9500e+001	0.0000e+000	
	8.7168e+002	4.5250e+001	0.0000e+000	
	8.7168e+002	5.1000e+001	0.0000e+000	
	8.7168e+002	5.6750e+001	2.4724e-002	
20	8.7168e+002	6.2500e+001	4.7216e-002	
	8.7168e+002	6.8250e+001	6.5532e-002	
	8.7168e+002	7.4000e+001	8.3563e-002	
	8.7168e+002	7.9750e+001	1.0154e-001	
	8.7168e+002	8.5500e+001	1.1797e-001	
25	8.7168e+002	9.1250e+001	1.3372e-001	
	8.7168e+002	9.7000e+001	1.4977 <b>e-</b> 001	
	8.7168e+002	1.0275e+002		
	8.7168e+002	1.0850e+002		
	8.7168e+002	1.1425e+002		
30	8.7168e+002	1.2000e+002	1.8264e-001	
	9.1500 <b>e</b> +002	5.0000e+000	0.0000e+000	
	9.1500e+002	1.0750e+001	0.0000e+000	
	9.1500e+002	1.6500e+001	0.0000e+000	
35	9.1500e+002	2.2250e+001	0.0000e+000	
	9.1500e+002	2.8000e+001		
	9.1500e+002	3.3750e+001	0.0000e+000	

	9.1500e+002	3.9500e+001	0.0000e+000
	9.1500e+002	4.5250e+001	0.0000e+000
	9.1500e+002	5.1000e+001	0.0000e+000
	9.1500e+002	5.6750e+001	4.5418e-003
5	9.1500e+002	6.2500e+001	2.7759e-002
	9.1500e+002	6.8250e+001	4.7768e-002
	9.1500e+002	7.4000e+001	6.730le-002
	9.1500e+002	7.9750e+001	8.5817e-002
	9.1500e+002	8.5500e+001	1.0198e-001
10	9.1500e+002	9.1250e+001	1.1834e-001
	9.1500e+002	9.7000e+001	1.3704e-001
	9.1500e+002	1.0275e+002	1.5430e-001
	9.1500e+002	1.0850e+002	1.6588e-001
	9.1500e+002	1.1425e+002	1.7281e-001
15 .	9.1500e+002	1.2000e+002	1.7788e-001
20	9.5832e+002	5.0000e+000	0.0000e+000
20	9.5832e+002	1.0750e+001	0.0000e+000
	9.5832e+002	1.6500e+001	0.0000e+000
	9.5832e+002	2.2250e+001	0.0000e+000
	9.5832e+002	2.8000e+001	0.0000e+000
	9.5832e+002	3.3750e+001	0.0000e+000
25	9.5832e+002	3.9500e+001	0.0000e+000
	9.5832e+002	4.5250e+001	0.0000 <b>e</b> +000
	9.5832e+002	5.1000e+001	0.0000e+000
	9.5832e+002	5.6750e+001	0.0000e+000
•	9.5832e+002	6.2500e+001	7.9093e-003
30	9.5 <b>832e</b> +002	6.8250e+001	2.9880e-002
	9.5832e+002	7.4000e+001	5.0797e-002
	9.5832 <b>e</b> +002	7.9750 <del>e+</del> 001	7.0155 <b>e-</b> 002
	9.5832e+002	8.5500e+001	8.7281e-002
- P	9.5832e+002	9.1250e+001	1.0473e-001
35	9.5832e+002	9.7000e+001	1.2446e-001
	9.5832e+002	1.0275e+002	1.4313e-001
	9.5832e+002	1.0850e+002	1.5665e-001

	9.5832e+002	1.1425e+002	1.6548e-001
	9.5832e+002	1.2000e+002	1.7204e-001
	1.0017e+003	5.0000e+000	0.0000e+000
5	1.0017e+003	1.0750e+001	0.0000e+000
	1.0017e+003	1.6500e+001	0.0000e+000
	1.0017e+003	2.2250e+001	0.0000e+000
	1.0017e+003	2.8000e+001	0.0000e+000
	1.0017e+003	3.3750e+001	0.0000e+000
10	1.0017e+003	3.9500e+001	0.0000e+000
	1.0017e+003	4.5250e+001	0.0000e+000
	1.0017e+003	5.1000e+001	0.0000e+000
	1.0017e+003	5.6750e+001	0.0000e+000
	1.0017e+003	6.2500e+001	0.0000e+000
15	1.0017e+003	6.8250e+001	1.2092e-002
•	1.0017e+003	7.4000e+001	3.4260e-002
	1.0017e+003	7.9750e+001	5.4766e-002
	1.0017e+003	8.5500e+001	7.3684e-002
	1.0017e+003	9.1250e+001	9.256le-002
20	1.0017e+003	9.7000e+001	1.1232e-001
	1.0017e+003	1.0275e+002	1.3101e-001
	1.0017e+003	1.0850e+002	1.4604e-001
	1.0017e+003	1.1425e+002	1.5707e-001
	1.0017e+003	1.2000e+002	1.6537e-001
25			
	1.0450e+003	5.0000e+000	0.0000e+000
	1.0450e+003	1.0750e+001	0.0000e+000
30	1.0450e+003	1.6500e+001	0.0000e+000
	1.0450e+003	2.2250e+001	0.0000e+000
	1.0450e+003	2.8000e+001	0.0000e+000
	1.0450e+003	3.3750e+001	0.0000e+000
	1.0450e+003	3.9500e+001	0.0000e+000
35	1.0450e+003	4.5250e+001	0.0000e+000
	1.0450e+003	5.1000e+001	0.0000e+000
	1.0450e+003	5.6750e+001	0.0000e+000

	1.0450e+003	6.2500e+001	0.0000e+000
	1.0450e+003	6.8250e+001	0.0000e+000
	1.0450e+003	7.4000e+001	1.7765e-002
	1.0450e+003	7.9750e+001	3.9637e-002
5	1.0450e+003	8.5500e+001	6.0243e-002
	1.0450e+003	9.1250e+001	8.0373e-002
	1.0450e+003	9.7000e+001	1.0032e-001
	1.0450e+003	1.0275e+002	1.1906e-001
	1.0450e+003	1.0850e+002	1.3510e-001
10	1.0450e+003	1.1425e+002	1.4793e-001
	1.0450e+003	1.2000e+002	1.5797e-001
	1.0883e+003	5.0000e+000	0.0000e+000
	1.0883e+003	1.0750e+001	0.0000e+000
15	1.0883e+003	1.6500e+001	0.0000e+000
•	1.0883e+003	2.2250e+001	0.0000e+000
	1.0883e+003	2.8000e+001	0.0000e+000
	1.0883e+003	3.3750e+001	0.0000e+000
	1.0883e+003	3.9500e+001	0.0000e+000
20	1.0883e+003	4.5250e+001	0.0000e+000
	1.0883e+003	5.1000e+001	0.0000e+000
	1.0883e+003	5.6750e+001	0.0000e+000
	1.0883e+003	6.2500e+001	0.0000e+000
	1.0883e+003	6.8250e+001	0.0000e+000
25	1.0883e+003	7.4000e+001	1.2775e-003
•	1.0883e+003	7.9750e+001	2.4559e-002
	1.0883e+003	8.5500e+001	4.6608e-002
	1.0883e+003	9.1250e+001	6.7829e-002
	1.0883e+003	9.7000e+001	8.8271 <b>e-</b> 002
30	1.0883e+003	1.0275e+002	1.0735e-001
	1.0883e+003	1.0850e+002	1.2423e-001
	1.0883e+003	1.1425e+002	1.3843e-001
	1.0883e+003	1.2000e+002	1.5002e-001

	1.1316e+003	5.0000e+000	0.0000e+000
	1.1316e+003	1.0750e+001	0.0000e+000
	1.1316e+003	1.6500e+001	0.0000e+000
	1.1316e+003	2.2250e+001	0.0000e+000
5	1.1316e+003	2.8000e+001	0.0000e+000
,	1.1316e+003	3.3750e+001	0.0000e+000
	1.1316e+003	3.9500e+001	0.0000e+000
	1.1316e+003	4.5250e+001	0.0000e+000
	1.1316e+003	5.1000e+001	0.0000e+000
10	1.1316e+003	5.6750e+001	0.0000e+000
10	1.1316e+003	6.2500e+001	0.0000e+000
	1.1316e+003	6.8250e+001	0.0000e+000
	1.1316e+003	7.4000e+001	0.0000e+000
	1.1316e+003	7.9750e+001	9.3618e-003
15	1.1316e+003	8.5500e+001	3.2700e-002
	1.1316e+003	9.1250e+001	5.4944e-002
	1.1316e+003	9.7000e+001	7.6071e-002
	1.1316e+003	1.0275e+002	9.5729e-002
	1.1316e+003	1.0850e+002	1.1341e-001
20	1.1316e+003	1.1425e+002	1.2876e-001
	1.1316e+003	1.2000e+002	1.4168e-001
		v	
	1.1750e+003	5.0000e+000	0.0000e+000
	1.1750e+003	1.0750e+001	0.0000e+000
25 .	1.1750e+003	1.6500e+001	0.0000e+000
	1.1750e+003	2.2250e+001	0.0000e+000
	1.1750e+003	2.8000e+001	0.0000e+000
	1.1750e+003	3.3750e+001	0.0000e+000
	1.1750e+003	3.9500e+001	0.0000e+000
30	1.1750e+003	4.5250e+001	0.0000e+000
	1.1750e+003	5.1000e+001	0.0000e+000
	1.1750e+003	5.6750e+001	0.0000e+000
	1.1750e+003	6.2500e+001	0.0000e+000
	1.1750e+003	6.8250e+001	0.0000e+000
35	1.1750e+003	7.4000e+001	0.0000e+000
	1.1750e+003	7.9750e+001	0.0000e+000
	1.1750e+003	8.5500e+001	1.8498e-002

	1.1750e+003	9.1250e+001	4.1753e-002
	1.1750e+003	9.7000e+001	6.3685e-002
	1.1750e+003	1.0275e+002	8.4069e-002
	1.1750e+003	1.0850e+002	1.0258e-001
5	1.1750e+003	1.1425e+002	1.1896e-001
	1.1750e+003	1.2000e+002	1.1090e-001

Table 3

5	The first	column	(X)	is	DNA Size	(Kilobases)	
	The second	d column	(Y)	is	Mobility	(cm/hr)	
	x	Y					
10	260	0.280					
	335	0.269					
	427	0.260					
	581	0.243					
	681	0.233					
15 .	774	0.219					
	832	0.209					
	913	0.200					
	1051	0.173					
	1600	0.129					
20	2200	0.100					
	2500	0.085					

### CLAIMS

1. A method for improving the separation of macromolecules in an eletrophoresis system, with the aid of a computer comprising the steps of:

entering a first size representing the smallest
macromolecule to be resolved;

entering a second size representing the largest
macromolecule to be resolved;

storing a knowledge base representative of calibration data obtained from separating macromolecules of known size under conditions which optimize separations;

testing said first size and said second size against said knowledge base, thereby generating a set of optimized parameters; and

calibrating said system with said optimized parameters, thereby optimizing macromolecule separation.

2. The method of claim 1, wherein said testing step comprises:

testing said first size and said second size against said knowledge base, thereby selecting an angle, a run distance, a voltage gradient, an initial switch time, a final switch time, and a run distance.

3. The method of claim 1 or claim 2, wherein said calibrating step comprises:

calibrating said system for optimal separation by using the angle, the run distance, the voltage gradient, the initial switch time, final switch time, and the run distance.

4. The method of any one of claims 1, 2 or 3, wherein said knowledge base storing step comprises:

storing a first plurality of relations between macromolecule sizes and switch times;

storing a second plurality of relations between macromolecule sizes and mobilities; and

storing a third plurality of relations between minimum macromolecule sizes, switch times and mobilities.

5. An electrophoresis system for improving the separation of macromolecules comprising:

an electrophoretic means;

a computer having a memory and a processor, said computer being selectively coupled to said electrophoretic means;

input means, coupled to said computer, for entering a first size representing the smallest macromolecule to be resolved and entering a second size representing the largest macromolecule to be resolved;

a knowledge base, coupled to said memory, for storing information representative of calibration data obtained from separating macromolecules of known size under conditions which optimize separations;

programming means, coupled to said memory, for instructing said processor to test said first size and said second size against said knowledge base, thereby generating a set of optimized parameters; and

means for calibrating said electrophoresis means with said optimized parameters, thereby optimizing macromolecule separation.

6. The system of claim 5, wherein said programming means comprises:

programming means, coupled to said memory, for instructing said processor to test said first size and said second size against said knowledge base, thereby selecting an angle, a run distance, a voltage gradient, an initial switch time, a final switch time, and a run distance.

7. The system of claim 5 or claim 6, wherein said means for calibrating comprises:

means for calibrating said system for optimal separation by using the angle, the run distance, the voltage gradient, the initial switch time, final switch time, and the run distance.

8. The system of any one of claims 5, 6 or 7 wherein said knowledge base comprises:

storage means, coupled to said memory, for storing a first plurality of relations between macromolecule sizes and switch times, a second plurality of relations between macromolecule sizes and mobilities, and a third plurality of relations between minimum macromolecule sizes, switch times and mobilities.

- 9. The system of any one of claims 5, 6, 7 or 8 further comprising Mapper means for receiving said optimised parameters, said Mapper means being selectively coupled to said computer through an external interface.
- 10. The system of any one of claims 5, 6, 7, 8 or 9 wherein said electrophoretic means is a CHEF electrophoretic device.

Patents Act 1977

xaminer's report to the Comptroller under Section 17 (The Search Report)

Application number

9120922.1

Relevant Technical fields

(i) UK CI (Edition K ) GIN (NBLE)

(ii) Int CI (Edition 5 ) BOID, CO7K, C25B, GOIN

Databases (see over)

(i) UK Patent Office

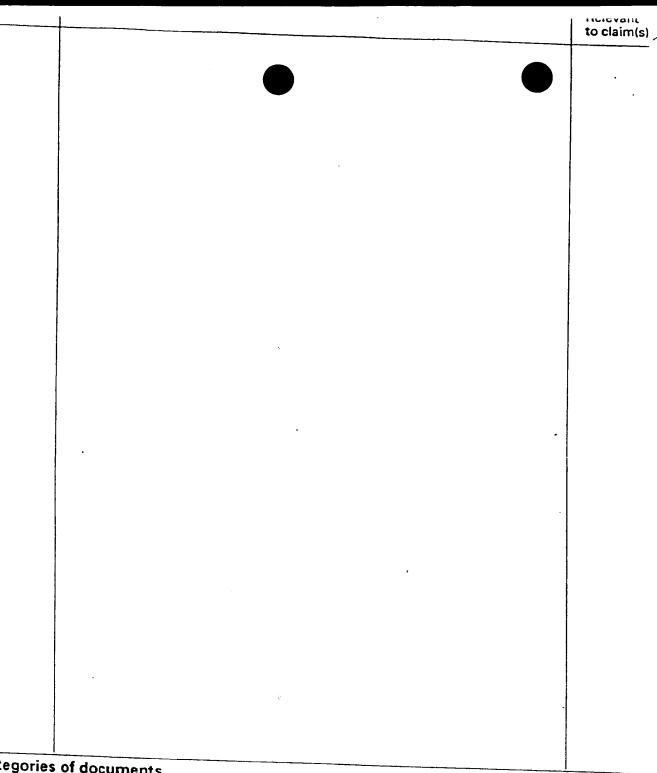
Databases: WPI

Databases: WPI

Documents considered relevant following a search in respect of claims

1-10

Identity of document and relevant passages	Relevant to claim(s)
EP 0395319 A1 (XEROX CORPORATION) SEE PARTICULARLY PAGE 6 LINES 6-10 AND PAGE 11 LINE 43 - PAGE 12 LINE 6	1, 5
	EP 0395319 A1 (XEROX CORPORATION) SEE PARTICULARLY PAGE 6 LINES 6-10 AND



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Document indicating lack of inventive step if mbined with one or more other documents of the me category.

Document indicating technological background d/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

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